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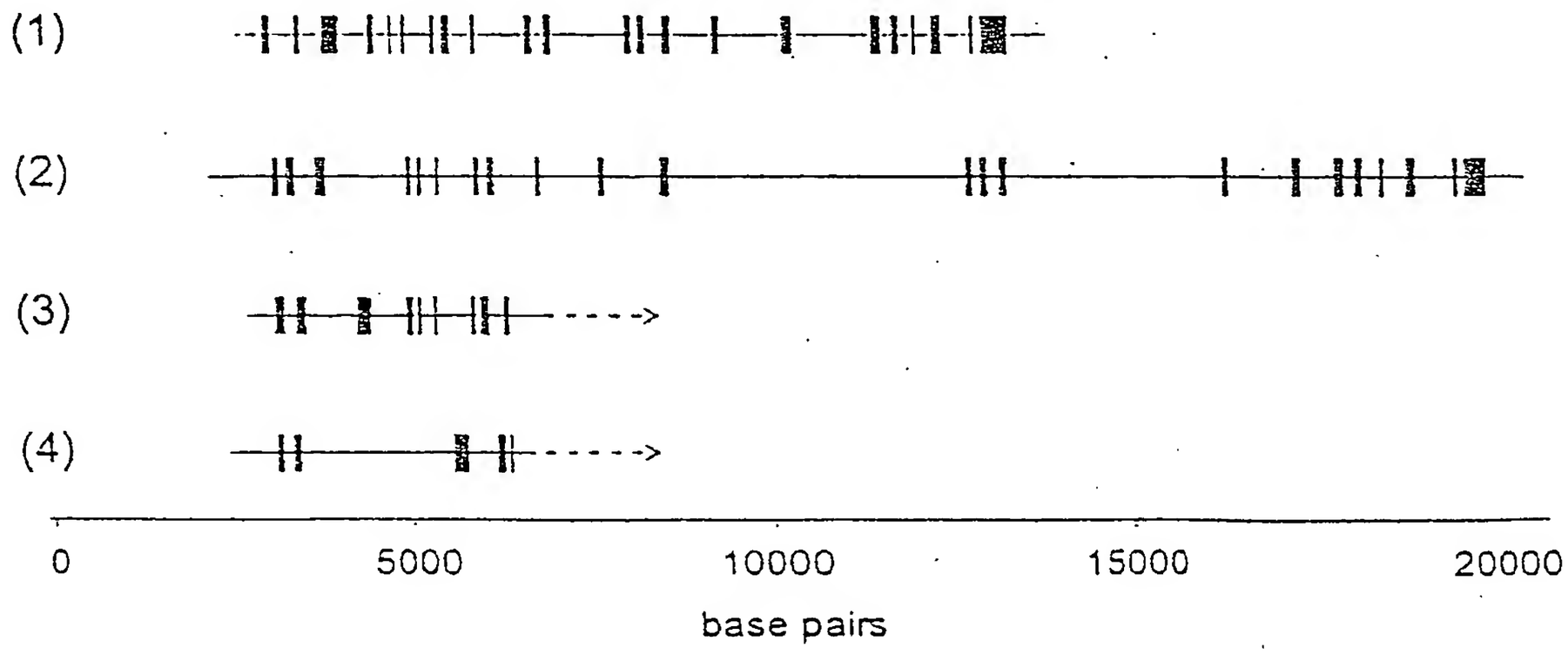
(54) Title: STARCH BRANCHING ENZYME



(57) Abstract: This invention relates to a new starch branching enzyme, and to the gene encoding the enzyme. In particular, the invention provides a new starch branching enzyme type II from wheat, the nucleic acid encoding the enzyme, and constructs comprising the nucleic acid. The invention also relates to a novel method for identification of branching enzyme type II proteins, which is useful for screening wheat germplasm for null or altered alleles of wheat branching enzyme IIb. The novel gene, protein and methods of the invention are useful in production of plants which produce grain with novel propertie example wheat grain containing high amylose or low amylopectin starch.

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STARCH BRANCHING ENZYME

This invention relates to a new starch branching enzyme, and to the gene encoding the enzyme. In particular, the invention relates to a new starch branching enzyme type II from wheat. The invention also relates to a novel method for identification of such branching enzyme type II proteins, which is useful for screening wheat germplasm for null or altered alleles of wheat branching enzyme IIb. The novel gene, protein and methods of the invention are useful in production of wheat plants which produce grain with novel properties for food and industrial applications, for example wheat grain containing high amylose or low amylopectin starch.

Background of the Invention

In plants, two classes of genes encode starch branching enzymes, known respectively as BEI, and BEII. In the monocotyledonous cereals, there is strong evidence demonstrating that the BEII class contains two independent types of genes, known in maize as BEIIa and BEIIb (Gao et al., 1996; Fisher et al., 1996). In barley, two types of genes have been reported, and shown to be differentially expressed (Sun et al., 1998). An additional class of branching enzyme (50/51 kD) from barley has also been described (Sun et al., 1996).

In dicotyledonous plants, loss of BEII activity through either mutation (Bhattacharyya et al., 1990) or gene suppression technologies gives rise to starches containing high amylose levels (Safford, 1998, Jobling 1999).

In monocotyledonous plants, mutations giving rise to high amylose contents are known in maize, rice and barley. In neither rice (Mizuno et al., 1993) nor barley (Schondelmaier et al., 1992) have the known high amylose phenotypes been associated with the BEIIa or BEIIb mutations respectively. However, in maize it is firmly

established that the high amylose phenotype is associated with down regulation of the BEIIb gene (Boyer et al., 1980; Boyer and Preiss, 1981, Fisher et al, 1996).

The impact of down-regulation of BEI has been investigated through antisense inhibition in potato tuber; the down-regulation has been found to alter the properties of the starch, but not its gross structural features, such as the amylose content (Filpse et al., 1996). In wheat, antisense down-regulation of BEI activity has small but significant effects on starch structure (Baga et al, 1999). The branching enzyme I gene from maize has been cloned (Kim et al., 1998), but mutants affecting branching enzyme I activity in maize are not known.

No mutations specifically reducing BEIIa activity have been reported, and no gene suppression experiments in plants have succeeded in reducing BEIIa activity, although the *du1* mutation in maize is known to reduce the expression of both BEIIa and starch synthase III. However, the *du1* mutation is now known to be due to mutation of the structural gene for starch synthase III (Gao 1998, Cao 1999).

In our previous patent application No. PCT/AU98/00743 (WO99/14314), we have described the structure of a BEII gene from wheat, which we have subsequently designated the BEIIa gene.

In the present application we describe the isolation of a second BEII gene from wheat, which we have designated the BEIIb gene, and discuss the uses to which this gene sequence can be applied. We have surprisingly found that in wheat the expression level of the various branching enzymes is very different to that in maize and barley. In this specification we show that BEIIb in wheat is expressed at low levels in the soluble fraction of the wheat endosperm, and is predominantly found within the starch granule. This indicates that there are important differences in the regulation of gene expression in wheat compared to other cereals, suggesting that the manipulation

of the amylose to amylopectin ratio in wheat will involve the manipulation of more than just the BEIIb gene.

We have also surprisingly found that the BEIIa and BEIIb gene structures are highly conserved with respect to exon size and position, allowing us to prepare DNA-based diagnostics which they can distinguish not only the BEIIa and BEIIb classes of genes, but also the forms of these genes encoded on the A, B and D genomes of wheat, and to identify the BEIIb proteins expressed by the wheat A, B and D genomes, providing an essential tool for the screening of wheat germplasm for null or altered alleles of wheat branching enzyme IIa.

Summary of the Invention

In a first aspect, the invention provides an isolated nucleic acid molecule encoding wheat starch branching enzyme IIb (BEIIb).

Preferably the nucleic acid sequence is a DNA sequence, and may be genomic DNA or cDNA.

Preferably the nucleic acid molecule has the sequence depicted in Figure 8 (SEQ ID NO:5), Figure 9 (SEQ ID NO:6), or SEQ ID NO:10. It will be clearly understood that the invention also encompasses nucleic acid molecules capable of hybridising to these sequences under at least low stringency hybridization conditions, or a nucleic acid molecule with at least 70% sequence identity to at least one of these sequences. Methods for assessing ability to hybridize and % sequence identity are well known in the art. Even more preferably the nucleic acid molecule is capable of hybridizing thereto under high stringency conditions, or has at least 80%, most preferably at least 90% sequence identity. A nucleic acid molecule having at least 70%, preferably at least 90%, more preferably at least 95% sequence identity to one or more of these sequences is also within the scope of the invention.

Biologically-active untranslated control sequences of genomic DNA are also within the scope of the invention.

Thus the invention also provides the promoter of BEIIb.

In a second aspect of the invention, there is provided a genetic construct comprising a nucleic acid sequence of the invention, a biologically-active fragment thereof, or a fragment thereof encoding a biologically-active fragment of BEIIb operably linked to one or more nucleic acid sequences which are capable of facilitating expression of BEIIb in a plant, preferably a cereal plant. The construct may be a plasmid or a vector, preferably one suitable for use in transformation of a plant. Such a suitable vector is a bacterium of the genus *Agrobacterium*, preferably *Agrobacterium tumefaciens*. Methods of transforming cereal plants using *Agrobacterium tumefaciens* are known; see for example Australian Patent No. 667939 by Japan Tobacco Inc.; Australian Patent No. 687863 by Japan Tobacco Inc.; International Patent Application No. PCT/US97/10621 by Monsanto Company; and Tingay *et al* (1997).

In a third aspect, the invention provides a genetic construct for targeting of a desired gene to endosperm of a cereal plant, and/or for modulating the time of expression of a desired gene in endosperm of a cereal plant, comprising a BEIIb promoter, operatively linked to a nucleic acid sequence encoding a desired protein, and optionally also operatively linked to one or more additional targeting sequences and/or one or more 3' untranslated sequences.

The nucleic acid encoding the desired protein may be in either the sense orientation or in the anti-sense orientation. Alternatively it may be a duplex construct, comprising a portion of the nucleic acid sequence encoding the desired protein in both the sense and anti-sense orientations, operably linked by a spacer sequence. It is contemplated that any desired protein which is encoded by a gene which is capable of being expressed in the endosperm of a cereal plant is suitable for use in the invention. Preferably the desired protein is an enzyme of the starch biosynthetic pathway. For example, the antisense sequences

of GBSS, starch debranching enzyme, SBE II, low molecular weight glutenin, or grain softness protein I, may be used. Preferred sequences for use in sense orientation include those of bacterial isoamylase, bacterial glycogen synthase, or wheat high molecular weight glutenin Bx17.

In a fourth aspect, the invention provides a wheat BEIIb polypeptide, comprising an amino acid sequence encoded by a nucleic acid molecule according to the invention, or a polypeptide having at least 70%, more preferably 80%, even more preferably 90% amino acid sequence identity thereto, and having the biological activity of BEIIb.

The polypeptide may be designed on the basis of amino acid sequences deduced from the nucleic acid sequences of the invention, or may be generated by expression of the wheat BEIIb nucleic acid molecule in a heterologous system. Suitable heterologous systems are very well known in the art, and the skilled person will readily be able to select a system suitable for the particular purpose desired.

In a fifth aspect, the invention provides an antibody directed against BEII polypeptide. The antibody may be polyclonal or monoclonal. It will be clearly understood that the invention also encompasses biologically-active antibody fragments, such as Fab, $(Fab)_2$, and ScFv. Methods for production of antibodies and fragments thereof are very well known in the art.

The antibodies of the invention may be used for identification and separation of BEIIb proteins, for example by affinity electrophoresis. This greatly facilitates the identification and combination of altered forms of BEIIb via analysis of germplasm, and greatly assists plant breeding. The antibodies of the invention are suitable for use in any affinity-based separation system, preferably using methods which overcome interference by amylases. Suitable methods are known in the art.

In a sixth aspect, the invention provides a plant cell

transformed by a genetic construct according to the invention, or a plant derived from such a cell. Additionally, a transformed plant cell may also comprise one or more null alleles for a gene selected from the group consisting of GBSS, BEIIa, and SSII. Preferably the plant is a cereal plant, more preferably wheat or barley.

In a seventh aspect, the invention provides a method of modifying the characteristics of starch produced by a plant, comprising the steps of:

a) increasing the level of expression of BEIIb in the plant, for example by introducing a nucleic acid molecule encoding BEIIb into a host plant, or

b) decreasing the level of expression of BEIIb in the plant, for example by introducing an anti-sense nucleic acid sequence directed to a nucleic acid molecule encoding BEIIb into a host plant.

As is well known in the art, over-expression of a gene can be achieved by introduction of additional copies of the gene, and anti-sense sequences can be used to suppress expression of the protein to which the anti-sense sequence is complementary. Other methods of suppressing expression of genes are known in the art, for example co-suppression, RNA duplex formation, or homologous recombination. It would be evident to the person skilled in the art that sense and anti-sense sequences may be chosen depending on the host plant, so as to effect a variety of different modifications of the characteristics of the starch produced by the plant.

Preferably the plant is a cereal plant, more preferably wheat or barley.

Preferably the branching of the amylopectin component of starch is modified by either of these procedures. More preferably a plant with high amylose content is produced.

In an eighth aspect, the invention provides a method of targeting expression of a desired gene to the endosperm of a cereal plant, comprising the step of transforming the plant with a construct according to the invention.

In a ninth aspect, the invention provides a method of identifying a null or altered allele encoding an enzyme of the starch biosynthetic pathway, comprising the step of subjecting DNA from a plant suspected to possess such an allele to a DNA fingerprinting or amplification assay, which utilizes at least one DNA probe comprising one or more of the nucleic acid molecules of the invention. The nucleic acid molecule may be a genomic DNA or a cDNA, and may comprise the full-length coding sequence or a fragment thereof. Any suitable method for identification of BEIIb sequences may be used, including but not limited to PCR, rolling circle amplification, RFLP, and AFLP. Such methods are well known in the art, and any suitable technique may be used.

In a tenth aspect, the invention provides a plant comprising one or more BEIIb null alleles, in combination with one or more other null alleles selected from the group consisting of BEIIa, GBSS, SSII and BEI. Optionally the plant may also comprise a BEIIa or BEIIb gene expressed in either the sense or the anti-sense orientation. The null alleles for BEIIa, GBSS SSII and BEI may be identified using methods described in PCT/AU97/00743.

It will clearly understood that the invention also encompasses products produced from plants according to the invention, including but not limited to whole grain, part grain, flour or starch.

Because of the very close relationship between *Aegilops tauschii*, formerly known as *Triticum tauschii*, and wheat, as discussed in PCT/AU97/00743, results obtained with *A. tauschii* can be directly applied to wheat with little if any modification. Such modification as may be required represents routine trial and error experimentation. Sequences from these genes can be used as probes to identify null or altered alleles in wheat, which can then be used in plant breeding programes to provide modifications of starch characteristics. The novel sequences of the invention can be used in genetic

engineering strategies or to introduce a desired gene into a host plant, or to provide anti-sense sequences for suppression of expression of the BEIIb gene in a host plant, in order to modify the characteristics of starch produced by the plant.

While the invention is described in detail in relation to wheat, it will be clearly understood that it is also applicable to other cereal plants of the family Gramineae, such as maize, barley and rice.

Methods for transformation of monocotyledonous plants such as wheat, maize, barley and rice and for regeneration of plants from protoplasts or immature plant embryos are well known in the art. See for example Lazzeri *et al*, 1991; Jahne *et al*, 1991 and Wan and Lemaux, 1994 for barley; Wirtzens *et al*, 1997; Tingay *et al*, 1997; Canadian Patent Application No. 2092588 by Nehra; Australian Patent Application No. 61781/94 by National Research Council of Canada, and Australian Patents No. 667939 and No. 687863 by Japan Tobacco Co.

The sequences of ADP glucose pyrophosphorylase from barley (Australian Patent Application No. 65392/94), starch debranching enzyme and its promoter from rice (Japanese Patent Publication No. Kokai 6261787 and Japanese Patent Publication No. Kokai 5317057), and starch debranching enzyme from spinach and potato (Australian Patent Application No. 44333/96) are all known.

Brief Description of the Figures

Figure 1 shows the sequence of the SBE9 branching enzyme cDNA encodes SBE IIa, cloned from a wheat cv Rosella cDNA library (SEQ ID NO:1).

Figure 2 shows the sequence of the branching enzyme BEIIa gene (SEQ ID NO:2) contained within the F2 lambda clone isolated from an *Aegilops tauschii* genomic DNA library.

Figure 3 shows the results of hybridisation of *Aegilops tauschii* DNA with probes derived from *wSBE II-DA1*

type sequences. A. Hybridisation with a probe from SBE9 consisting of exons 5-9. B. Hybridisation with fragment F2.2 (consisting of exons 4-9 and introns 4-8 and part of introns 3 and 9). Enzymes used for the digest were: 1. *Bam* HI, 2. *Dra* I, 3. *Eco*R I, 4. *Eco*R V. Molecular size markers are indicated.

Figure 4 shows the alignment of sequences of Intron 5 fragments from the A, B and D genomes of wheat

Figure 5 shows the PCR analysis of *A. tauschii* genomic clones using Intron V sequences.

Figure 6 shows the alignment of a 262bp PCR fragment amplified from hexaploid wheat using the primers sr913F and WBE2E6R, and a region from the wheat branching enzyme IIb gene *wSBE II-DB1*.

Figure 7 shows the alignment of barley branching enzyme IIb cDNA, wheat branching enzyme IIb cDNA, and SBE9 sequences with the sequence of the wheat (*A. tauschii*) branching enzyme IIb gene.

Figure 8 shows the partial genomic sequence of a branching enzyme IIb gene from *A. tauschii* (SEQ ID NO:5).

Figure 9 shows the sequence of a cDNA for branching enzyme IIb gene from hexaploid wheat (SEQ ID NO:6).

Figure 10 shows the sequence alignment of branching enzyme genes. The cDNA sequences used for this analysis were SBE9 (SEQ ID NO:1; Figure 1), wheat BEIIb cDNA (SEQ ID NO:6; Figure 9), Y11282, a wheat branching enzyme sequence (Nair et al. 1997), barley BEIIa (Sun et al. 1998), barley BEIIb (Sun et al. 1998), rice BEIII (Mizuno et al. 1993), rice BEIV (Genbank Accession No. E14723) maize BEIIa (Gao et al. 1997) and maize BEIIb (Gao et al. 1997). The observed N-terminal of wheat (Morell et al., 1997; Y11282) is shown in bold. Figure 11 shows the dendrogram of BE sequences. .The sequences analysed were for wheat Y11282 (Nair et al., 1997), SBE 9 (SEQ ID NO:1; (Figure 1), wheat BEIIb (SEQ ID NO:9; Figure 9), barley IIa and IIb (Sun et al. 1998), maize IIa (Gao et al. 1997), maize IIb (Fisher et al. 1993), rice III (Mizuno et al. 1993), rice IV

(Genbank accession E14723), potato BEI (Khoshnoodi et al. 1997), potato BE II(Cangiano et al 1993), pea BEI and BEII (Burton et al.1995), E.coli BE (Baecker et al. 1986) and bacillus (Kiel et al 1992). Note that pea BE I and pea BE II sequences correspond to maize BE II and BE I respectively because of differences in nomenclature conventions.

Figure 12 shows the comparison of exon/intron structure for the BEIIa and BEIIb genes. (1) wheat branching enzyme IIa gene, wSBE II DA1 (2) maize amylose extender BEIIb gene (3) partial wheat branching enzyme IIb gene, wSBE II DB1 (4) partial barley branching enzyme IIb gene.

Figure 13 shows the results of analysis of the expression of mRNA for the BEIIa and BEIIb genes in wheat. Panel (A): Hybridisation of SBE9 probe to lanes 1 to 3 and hybridisation of wheat BEIIb cDNA probe to lanes 4 to 6. Panel (B): mRNA loading for each lane.

Lanes 1 and 4 contain leaf mRNA; lanes 2 and 5 contain pre-anthesis floret mRNA; lanes 3 and 6 contain mRNA from wheat endosperm collected 15 days after anthesis.

Figure 14 shows the results of analysis of wheat endosperm branching enzyme IIa by affinity electrophoresis.

Samples: Lanes 1,4 and 7 contained 20 µg endosperm soluble protein, lanes 2, 5 and 8 contained 30 µg endosperm soluble protein and lanes 3 and 6 contained 10 µg endosperm soluble protein.

Figure 15 shows the results of non-denaturing gel electrophoresis analysis of branching enzymes in the soluble fraction of wheat endosperm.

Samples were: Lane 1, R6 pre-immune, 1:100; Lane 2, R6 pre-immune, 1:3000; Lane 3, R6, 1:100; Lane 4, R6, 1:1000; Lane 5, R6, 1:3000; Lane 6, 3KLH, 1:2000; Lane 7, 3KLH, 1:5000; Lane 8, R7 pre-immune, 1:1000; Lane 9, R7 pre-immune 1:5000; Lane 10, R7, 1:1000; Lane 11, R7, 1:3000; Lane 12, R7, 1:5000

Figure 16 shows the results of affinity

electrophoresis separation of branching enzyme IIa forms from diverse wheat germplasm using the gel conditions described in Figure 11 (Panel C). Panel A. Lane 1, Durati, *T. durum*; Lane 2 *A. tauschii*, Accession No. 24242; Lane 3, *A. tauschii*, Accession No. 24095; Lane 4, *A. tauschii*, Accession No. 24092; Lane 5, Hartog, *Triticum. aestivum*; Lane 6, Rosella, *T. aestivum*; Lane 7, Corrigin, *T. aestivum*; Lane 8, Bodallin, *T. aestivum*; Lane 9, Beulah, *T. aestivum*; Lane 10 Bindawarra, *T. aestivum*; Lane 11, Barley (*Hordeum vulgare*). Panel B. Lane 1: Afghanistan 006, *Triticum durum*; Lane 2, Persia 20, *T. aestivum*; Lane 3, Afghanistan 8, *T. aestivum*; Lane 4, Kashmir 4, *T. aestivum*; Lane 5, Gandum Sockhak, *T. aestivum*; Lane 6, Warbler, *T. aestivum*; Lane 7, Bayles, *T. aestivum*; Lane 8, Kometa; Lane 9, Kashmir 14, *T. aestivum*; Lane 10, Rosella, *T. aestivum*; Lane 11, Kashmir 8, *T. aestivum*; Lane 12, Beijing 10, *T. aestivum*; Lane 13, Savannah, *T. aestivum*; Lane 14, Afghanistan 55-623, *T. aestivum*; Lane 15, Karizik, *T. aestivum*; Lane 16, Indore E98, *T. durum*; Lane 17, Iraq 17, *T. durum*; Lane 18, Seri 82, *T. aestivum*; Lane 19, Indore 19, *T. aestivum*.

Figure 17 shows the results of two-dimensional separation of the components of the wheat starch granule 88 kD band. The wheat starch granule 88 kDa band was electrophoresed in the first dimension through an SDS-PAGE gel. Lanes were excised, renatured, and placed on top of a non-denaturing PAGE gel and electrophoresed ina second dimension. Two lanes were placed on top of each non-denaturing PAGE gel. (A) protein staining with Coomassie blue reagent (B) Immunoblotting of gels with either 3KLH or R6 antibodies, as indicated on the figure.

Figure 18 is a diagrammatic representation of the BEII genes from various species, showing the exon/intron structure. The dark rectangles represent exons.

Figure 19 shows the results of PCR amplification of SBE IIb gene from CS nullisomic lines, using the primers ARA 12F and ARA 10R.

Figure 20 shows the results of PCR amplification of SBE IIb gene, using the primers ARA 6F and ARA 8R from *Triticum* spp. Lanes: 1) *T. monococcum*, 2) *T. durum*, 3) *T. urartu*, 4) *T. tauschii*, 5) CSDT2DS, 6) CSDT2BL-9, 7) CSDT2AS and 8) CS.

Figure 21 shows the alignment of the exon 1 - intron 1 - exon 2 region of the SBE IIb gene from the A, B and D genomes. * indicates that the sequence could not be specifically assigned to the A or B genome.

Figure 22 shows the alignment of the BEIIb sequences from each genome.

Figure 23 shows the results of PCR amplification of the SBE IIb gene was carried out using the primers ARA 19F and ARA 15R, followed by restriction digestion using *Rsa*1. Lanes 1) CS, 2) *T. monococcum*, 3) *T. tauschii*, 4)CSDT2BL-9, which is missing part of the long arm of chromosome 2B, and 6) CSDT2AS, which is missing the long of chromosome 2A.

Figure 24 shows the results of PCR amplification of intron 3 region of SBE IIb from wheat lines, using the primers ARA 19F and ARA 23R followed by *Rsa* 1 digestion. Lane 12 is the null mutant for the D genome

Figure 25 is a schematic representation showing the development of the SBE IIa construct. A) Biogemma vector, pDV03000; B) pBluescript carrying the full length cDNA of SBE IIa; C) SBE IIa construct in pDV03000; D) Sense IIa construct and E) Antisense IIa construct.

Figure 26 is a schematic representation of the development of the SBE IIb construct. A) Biogemma vector, pDV03000; B) pGEM-T carrying a 1046bp fragment of SBE IIb; C) SBE IIb construct in pDV03000; D) Sense IIb construct and E) Antisense IIb construct.

Figure 27 is a schematic representation of a SBE II duplex construct. A) SBE sequence inserted in between the promoter and the terminator in its linear form; B) Duplex formation of mRNA within the transgenic plant.

Example 1 *Isolation of BEII genes from an A. tauschii genomic library and their characterisation by PCR*

Plant material

Aegilops tauschii, CPI 110799, was used for the construction of the genomic library. Previously this accession has been shown to be most like the ancestral D genome donor of wheat, on the basis of the conservation of order of genetic markers (Lagudah et al. 1991). The *Triticum aestivum* cultivars Rosella, Wyuna and Chinese Spring were used for the construction of different cDNA libraries.

cDNA and genomic libraries

The construction of the cDNA and genomic libraries used in this example was as described in Rahman et al., (1997,1999) and in Li et al. (1999). Conditions for library screening were hybridisation at 25% formamide, 5XSSC, 0.1% SDS, 10X Denhardts, 100µg/ml salmon sperm DNA at 42°C for 16h, followed by washing at 2XSSC, 0.1%SDS at 65°C for 3X1h.

Screening of a wheat cDNA library

Screening of a wheat cv Rosella cDNA library prepared from endosperm (mid-stage of development) with the maize SBE I clone (Baba et al., 1991) at low hybridisation stringency led to the isolation of two classes of positive plaques. One class hybridised strongly to the probe, and encoded wheat SBE I (Rahman et al., 1997,1999). The second class was weakly hybridising. The clone with the longest insert from this second class was called SBE 9, and its sequence showed greater identity to SBE II than to SBE I type sequences. This was designated SBE IIa. The sequence of SBE 9 (SEQ ID NO:1) is set out in Figure 1.

Screening of A. tauschii genomic library

A genomic library constructed from *A.tauschii* was screened by DNA hybridisation with SBE9, and four positive clones were purified. These were designated F1 to F4. The sequence from positions 537 to 890 of SBE9 was amplified by PCR, and used to screen the *A. tauschii* library again. Clones isolated from this screening are referred to as G1 and G2 and H1 to H8

(1) Number of *BEII* type genes in wheat

The sequence of a branching enzyme gene, designated F2, from *Aegilops tauschii* was described in WO99/14314, and is given in Figure 2 (SEQ ID NO:2). A probe generated from F2, designated F2.2, contained sequences from 2704 to 4456 bp of SEQ ID NO:2, and contained exons 4-9, introns 4-8, and parts of intron 3 and 9. Hybridisation of *A. tauschii* DNA (cut with four different restriction enzymes) with F2.2 revealed only one strongly hybridising band and several very faint bands (Figure 3, panel B), consistent with the presence of a single BEII type gene in the *A. tauschii* genome. The cDNA clone, SBE9 (SEQ ID NO:1) has >95% identity to the exon regions of the F2 branching enzyme gene. A region of SBE9 from nucleotides 537 to 890, including exons 5 to 9, was used as a hybridisation probe, and gave a much more complex pattern (Figure 3, panel A), strongly indicating that there is more than one BEII gene type in the *A. tauschii* genome.

Example 2: *PCR analysis of BEIIa - Intron 5*

PCR primers, sr913F (5' ATC ACT TAC CGA GAA TGG G 3', SEQ ID NO:3) and WBE2E6R (5' CTG CAT TTG GAT TTC AAT TG 3', SEQ ID NO:4) were designed to anneal to Exon 5 and Exon 6 respectively of the wheat F2 gene in order to amplify the intron region (Intron 5) between these exons. Analysis of the products of PCR reactions using these primers shows that the primers amplify fragments of 228 bp from the A-genome of wheat, 226 bp from the D genome and 217 bp from

the B genome. These fragments were shown to be amplified from chromosome 2A, 2D and 2B of wheat respectively by analysis of nullisomic/tetrasomic chromosome-engineered lines of wheat. In addition to these fragments, a 262 bp fragment was amplified, and this fragment (designated the 262 bp Universal fragment) was not polymorphic among the chromosome engineered lines tested. The 262 bp Universal fragment and the A, B and D regions from the F2 gene were cloned and sequenced, and the sequence comparison is shown in Figure 4.

Example 3: *Classification of the G1-G2 and H1-H10 genes*

PCR analysis using PCR primers sr913F (5' ATC ACT TAC CGA GAA TGG G 3') and WBE2E6R (5' CTG CAT TTG GAT TTC AAT TG 3') showed that the H1 to H10 lambda clones yielded an approximately 200 bp fragment, and the G1 and G2 clones yielded an approximately 260 bp fragment (Figure 5). Partial sequencing of G1 and G2 showed that the parts of the sequence analysed had 80% identity with the exons 4 and 5 of *wSBE II-DA1*, but the intervening intron contained a sequence that showed no homology to any sequence contained within F2.

However, the G1 and G2 clones from *A. tauschii* showed 92.7% identity to the sequence of the 262 bp universal fragment amplified and cloned from hexaploid wheat, and an alignment of these sequences is shown in Figure 6. Figure 7 shows an alignment of a region corresponding to the 537 to 890 bp region of the SBE9 clone from the cDNAs for barley BEIIb (Sun et al., 1995, Sun et al., 1998), SBE9, wheat BEIIb cDNA with the sequence from clone G1. Further sequencing of G1 led to the isolation of a sequence, shown in Figure 8 (SEQ ID NO:5), which showed high identity with the sequence reported by Sun et al.(1998) for the 5' end of barley IIb cDNA and the partial sequence for the cognate gene. G1 and G2 therefore contain a gene which is distinct from F2, and which has high homology to barley BEIIb. We have designated this gene *wSBE II-DB1*.

Example 4: *Isolation of a wheat BEIIb cDNA and an additional genomic fragment*

A barley cDNA library was constructed using 5 µg of polyA$^+$ mRNA (1.67 µg of polyA$^+$ mRNA from 10, 12 and 15 DPA endosperm tissues were pooled). cDNA was synthesised using the cDNA synthesis system marketed by Life Technology, except that the NotI-$(dT)_{18}$ primer (Pharmacia Biotech) was used to synthesise the first strand of cDNA. Pfu polymerase was added to the reaction after second strand synthesis to flush the ends of cDNAs. SalI-XhoI adapter (Stratagene) was then added to the cDNAs. cDNAs were ligated to SalI-NotI arms of λZipLox (Life Technology) after digestion of cDNAs with NotI followed by size fractionation (SizeSep 400 spun Column of Pharmacia Biotech). The entire ligation reaction (5 µl) was packaged using Gigapack III Gold packaging extract (Stratagene). The titre of the library was tested by transfecting either the Y1090(ZL) or the LE392 strain of *E.coli*.

Primers 1 and 2 (Sun et al. 1998)), were used for PCR amplification of a fragment from a barley cDNA library (Ali et al., 2000) using conditions described in Sun et al. (1998). The identity of this fragment was confirmed by sequence analysis, and the fragment was used as a probe to isolate a cDNA by hybridisation, cDNA from a cDNA library constructed from Chinese Spring (Li et al. 1999).

This cDNA was designed wBEIIb, and its sequence is shown in Figure 9 (SEQ ID NO:6). This probe was also used to reprobe the genomic library from *A.tauschii* referred to above, and a clone, designated G5, was recovered from this screen. Analysis showed that the wBEIIb cDNA sequence showed 98.5% identity and the G5 sequence showed 100% identity to sequences already recovered from G1 and G2. G5 therefore represented the same *wSBE II-DB1* gene, and the wBEIIb cDNA is a product of the orthologous gene in hexaploid wheat.

Example 5: *Relationships between BEII sequences*

Deduced amino acid sequences for branching enzymes from various cereals were analysed using the PILEUP program from the GCG suite of programs (Devereux 1984), and an alignment of these sequences is shown in Figure 10. The PILEUP analysis used a penalty of 12 for insertion of a gap and 0.1 for extending the gap per residue. The cDNA sequences used for this analysis were SBE9 (SEQ ID NO:1; Figure 1), wheat BEIIb cDNA (SEQ ID NO:6; Figure 9), Y11282, a wheat branching enzyme sequence (Nair et al.1997), barley BEIIa (Sun et al. 1998), barley BEIIb (Sun et al. 1998) , rice BEIII (Mizuno et al. 1993), rice BEIV (Genbank Accession No. E14723) maize BEIIa (Gao et al. 1997) and maize BEIIb (Fisher et al., 1993). The observed N-terminal of wheat (Morell et al., 1997; Y11282) is shown in bold.

The relationships between branching enzyme sequences are illustrated in Figure 11, using a dendrogram generated by the PILEUP program. The sequences analysed were for wheat Y11282 (Nair et al., 1997), SBE 9 (Figure 1), wheat BEIIb (Figure 9), barley IIa and IIb (Sun et al. 1998), maize BEI (Kim et al, 1998), maize IIa (Gao et al. 1997), maize IIb (Fisher et al. 1993), *Arabidopsis* BEII (U22428, Fisher et al., 1996), *Arabidopsis* BEII (U18817, Fisher et al., 1996), rice I (Kawasaki et al., 1993), rice III (Mizuno et al. 1993), rice IV (Genbank accession E14723), potato BEI (Khoshnoodi et al. 1997), potato BE II (Cangiano et al 1993), pea BEI and BEII (Burton et al.1995), *E. coli* BE (Baecker et al. 1986) and bacillus (Kiel et al 1992). Note that pea BE I and pea BE II sequences correspond to maize BE II and BE I respectively because of differences in nomenclature conventions.

On the basis of this comparison, the branching enzyme gene contained on clone F2 was classified as a BEIIa type gene and designated *wSBE II-DA1*.

Example 6: *Structure of the wSBE II-DA1 and wSBE II-DB1 genes*

Figure 12 shows a comparison of the exon/intron structures of the wheat wSBE II-DA1 and wSBE II-DB1 genes. The structure of the *wSBE II-DB1* gene is shown from the beginning of the wheat BEIIb cDNA through to exon 5. Hybridisation results suggest that regions at the 3' end of the wheat BEIIb cDNA are not contained within any of the clones G1,G2 and G5. This is not surprising, as the maize SBE II b gene extends over 16.5kb and required the isolation of two genomic clones (Kim et al 1998). The positions of the intron/exon boundaries for the first five introns of the wheat BEIIa and BEIIb genes are conserved, as shown in Table 1. The size of the first five introns in *wSBE II-DB1* vary considerably in size from the first five introns in *wSBE II-DA1*.

Table 1

Exon/Intron Structures of Cereal branching Enzyme Genes

Exons				
	Wheat wSBE II-DA1	Maize BEIIb	Wheat WSBE II-DB1	Barley BEIIb
1	123[a]	112[a]	148[a]	121[a]
2	98	146	146	152
3	242	155	230	230
4	99	99	99	99
5	43	43	43	43[b]
6	60	60	60	
7	81	81	81	
8	117	117	117	
9	81	84	84	
10	122	122		
11	120	120		
12	130	130		
13	111	111		
14	129	129		
15	104	104		
16	145	145		
17	148	148		
18	105	101		
19	74	78		
20	156	156		
21	75	75		
22	384	84		

Introns				
	Wheat wSBE II-DA1	Maize BEIIb	Wheat WSBE II-DB1	Barley BEIIb
1	327	106	148	105
2	276	244	663	2064
3	401	1086	465	388
4	169	76	74	74
5	152	196	181	
6	335	499	442	
7	83	81	79	
8	288	567	178	
9	629	775		
10	175	751		
11	974	4020		
12	88	86		
13	201	148		
14	579	3051		
15	841	872		
16	1019	457		
17	135	144		
18	176	226		
19	201	266		
20	377	448		
21	89	96		

[a]. Exon 1 numbering begins from ATG of translation start codon
[b]. Partial sequence for exon or intron

Example 7: *Expression analysis at the mRNA level*

RNA from endosperm at different developmental stages was obtained from wheat grown in the glasshouse as described in Li et al. (1999). RNA was extracted by the method of Higgins et al. (1976), separated on denaturing formamide gels and blotted onto Hybond N+ paper, essentially as described in Maniatis et al. (1992). Probes were prepared from the extreme 3' ends of SBE9 (bases 2450 to 2640 of SEQ ID NO:1) and wBEIIb cDNA (bases 2700 to 2890 of SEQ ID NO:6) by PCR using the following scheme: 94°C, 2min, 1 cycle, 94°C, 30s, 55°C, 30s, 72°C, 30s, 36 cycles, 72°C 5min, 1cycle, 25°C, 1min, 1cycle. The probes were from the 3' untranslated region, and were specific for either *wSBE II-DA1* or *wSBE II-DB1* type sequences. An RNA species of about 2.9kb hybridised to each probe (Figure 13 Panel B). However, the intensity of hybridisation determined by densitometry, and normalised for differences in RNA loading), indicated that RNA hybridising to the *wSBE II-DB1* gene was present at 2.5 to 3 fold lower concentration than RNA hybridising to the *wSBE II-DA1* gene.

Example 8: *Analysis of branching enzymes by affinity electrophoresis demonstrates that only BEIIa is predominant in the soluble fraction*

In Morell et al., (1997), we reported that only a single form of branching enzyme II could be identified in the wheat developing endosperm soluble fraction. However, this was on the basis of anion-exchange chromatography, and it remained possible that there were multiple forms, even though they could not be separated by this technique. Matsumoto has developed an affinity electrophoresis method for measuring the interaction of branching enzymes with polysaccharide substrates (Matsumoto et al., 1990), and we have further developed this technique specifically to allow the separation of the branching enzyme IIa forms encoded by each of the three wheat genomes. Figure 14 shows an

immunoblot of a non-denaturing polyacrylamide gel electrophoresis experiment in which the gel matrix contained the β-limit dextrin of maize amylopectin alone (Figure 14, lanes 1 and 2), showing separation of three forms of branching enzyme IIa. Resolution is slightly enhanced by the addition of the α-amylase inhibitor acarbose (Figure 14, lanes 3,4 and 5), and substantially enhanced by the addition of α-cyclodextrin (Figure 14 lanes 6, 7 and 8).

A non-denaturing gel was prepared, containing a stacking gel composed of 0.125 M Tris-HCl buffer (pH 6.8), 6% acrylamide, 0.06% ammonium persulphate and 0.1% TEMED. The separating gel was composed of three panels. The basic non-denaturing gel mix contained 0.34 M Tris-HCl buffer (pH 8.8), CHAPS (0.05%), glycerol (10.3%), acrylamide (6.2%), 0.06% ammonium persulphate, 0.1% TEMED and the β-limit dextrin of maize amylopectin (0.155%). Panel A (lanes 1 and 2) contained only the basic non-denaturing gel reagents. Panel B (Lanes 3, 4 and 5) contained the basic non-denaturing gel reagents and 0.066 mM acarbose. Panel C (lanes 6, 7 and 8) contained the basic non-denaturing gel reagents and 0.067 mM α-cyclodextrin.

Following electrophoresis at 100 V for 16 hours at 4 ºC, the proteins in the separating gel were transferred to nitrocellulose membrane according to Morell et al (1997) and immunoreacted with 1:5000 dilution of 3KLH antibodies (raised against the synthetic peptide AASPGKVLVPDESDDLGC (SEQ ID NO:7) coupled to the keyhole limpet hemocyanin via the heterobifunctional reagent m-Maleimidobenzoyl-N-hydroxysuccinimide ester).

The use of a β-limit dextrin provides a superior separation because it prevents interference with the separation by endogenous β-amylases in the wheat endosperm tissue, and the use of α-cyclodextrin in the assay further enhances the separation. Without wishing to limit the invention by any proposed mechanism, we believe that this enhancement may result from the inhibition of endogenous

wheat endosperm α-amylases.

The analysis shows that three branching enzyme II proteins are present, and that each of these proteins cross-reacts with antibodies to a synthetic oligopeptide designed from the N-terminal region of the BEIIa protein in a region that shares no homology with the wheat BEIIb protein.

The soluble fraction of the wheat endosperm was reacted with various antibodies raised against peptides designed on the basis of the sequences of the wheat BEIIa (see Figure 12) or the wheat BEIIb cDNA. Figure 15 shows that only 3KLH, raised against the N-terminus of BEIIa, cross-reacted with proteins (marked by arrows) in the soluble fraction which show a specific shift in mobility in the presence of the β-limit dextrin of amylopectin and α-cyclodextrin. Gels were prepared as described in Figure 14, except that the gel used in Panel A contained the non-denaturing gel mix without the β-limit dextrin of maize amylopectin. Panel B contained the non-denaturing gel mix plus α-cyclodextrin. An extract of developing wheat endosperm was prepared using 3 volumes of extraction buffer per g of tissue, and 140 µl of sample applied per gel. Following electrophoresis at 100 V for 16 hours at 4 ºC, the proteins in the separating gel were transferred to nitrocellulose membrane according to Morell et al (1997) which was cut into 1 cm strips. The antibodies prepared were 3KLH (see Figure 11), R6 (raised in rabbit against the synthetic peptide AGGPSGEVMIGC (SEQ ID NO:8) coupled to the keyhole limpet hemocyanin via the heterobifunctional reagent m-Maleimidobenzoyl-N-hydroxysuccinimide ester); pre-immune serum from the R6 rabbit; R7 (raised in rabbit against the synthetic peptide GGTPPSIDGPVQDSDGC (SEQ ID NO:9) coupled to the keyhole limpet hemocyanin via the heterobifunctional reagent m-maleimidobenzoyl-N-hydroxysuccinimide ester) and pre-immune serum from the R7 rabbit.

As in Figure 14, the BEIIa protein is separated into three forms (indicated by arrows in Figure 15, Panel B), by affinity electrophoresis in the presence of β-limit dextrin. In barley (Sun et al., 1997) and maize (Bayer and Preiss 1981) both branching enzymes IIa and IIb are present in the soluble fraction. In some subsequent experiments we have detected low levels of BE IIb in the soluble fraction.

The separation of the forms of BEIIa encoded by each wheat genome is demonstrated in Figure 16. In Panel (A) the diploid *A. tauschii* (lanes 2,3 and 4) and barley line (lane 11) yields a single band. However, the tetraploid *T. durum* lines (Panel A lane 1, Panel B, lanes 1, 16, and 17) and hexaploid *T. aestivum* lines (Panel A lanes 5-10, Panel B lanes 2-15, 18-19) give at least 2 bands. Some hexaploid lines (panel A, lane 7 and 9, Panel B lanes 2-6, lanes 8-9, lane 13) yield 2 bands, indicating either that they are null for one genome or that the products of two genomes migrate with identical mobility in the gel system.

The use of the separation system as a means of screening for wheat genomes with altered or null alleles of branching enzyme IIa is demonstrated by Figure 14 (Panel B), where different lines are shown to have different numbers and mobilities of branching enzyme IIa proteins.

Example 9: *Presence of two classes of proteins in the starch granule at 88 kDa and their differential antibody binding.*

The wheat starch granule contains a number of proteins that have been analysed by SDS-PAGE (Rahman et al., 1995, Denyer et al., 1995, Takaoka et al, Li et al., 1999a, Li et al, 1999b) and two-dimensional gel electrophoresis (Yamamori and Endo, 1996). The following bands have been identified: 60 kDa, GBSS; 75 kDa, SSI; 100 kDa, 108 kDa and 115 kDa, SSII). An 88 kDa band is also observed, and has been shown to be associated with branching enzyme activity (Denyer et al., 1995) and to react to antibodies to maize BEII (Rahman et al., 1995). This protein band was shown to

contain at least two protein components.

This analysis has been extended by purification and analysis of the individual granule proteins. The granule proteins were isolated from 4.7g of wheat starch granules by boiling in 24 ml of SDS buffer (50 mM Tris-HCl buffer pH 6.8, 10% SDS and 6.25% 2-mercaptothanol) as described by Rahman et al., (1995). Residual granular starch was removed by centrifugation, and granule proteins were separated by applying the supernatant to a 9% SDS-PAGE gel prepared in a Biorad Model 491 Prep Cell apparatus. The SDS gel contained a stacking gel composed of 0.125 M Tris-HCl buffer (pH 6.8), 0.25% SDS, 6% acrylamide, 0.06% ammonium persulphate and 0.1% TEMED and a separating gel containing 0.34 M Tris-HCl buffer (pH 8.8), 0.25% SDS, acrylamide (9 %), 0.06% ammonium persulphate, and 0.1% TEMED. The samples were electrophoresised at 60 mAmp constant current for 16 hours, and fractions of ractions (5 ml) collected by a pump operating at 0.5 ml/min. Fractions were analysed by SDS-PAGE, and fractions containing an 88 kDA band precipitated by the addition of 3 volumes of acetone. The precipitate from each 5 ml fraction was collected by centrifugation, the sample dissolved in SDS buffer, and electrophoresed through a standard 8% SDS-PAGE gel. The lane was excised from the gel and renatured in 0.04 M Tris for 2 hours. To generate a two-dimensional separation, the gel was then laid across the top of a second non-denaturing PAGE gel and electrophoresed. Proteins were identified by staining with Coomassie blue (a 50:50 mixture of 2.5% Coomassie Blue R-250 and Coomassie Blue G250 solutions).

Figure 17, Panel (A) shows that two proteins were visible after staining, and these were designated 88 kD (U) and 88 kD (L), as indicated by the arrows. Immunoblotting of the two-dimensional gel with peptide antibodies to the N-terminal of BEIIa (3KLH) and to the N-terminus of the wheat BEIIb cDNA sequence (R6; see Figures 12 and 13 for details of the antibodies are set out in Example 8)

indicated preferential binding of the R6 antibody to 88 kD (U) and preferential binding of 3KLH to 88 kD (L) (Figure 17, Panel B), providing a provisional assignment of these proteins as BEIIb and BEIIa respectively.

The proteins were further analysed by digestion with trypsin, and the peptides released were identified by MALDI-TOF analysis at the Australian Proteome Analysis Facility, Macquarie University, Sydney. The results of this analysis, shown in Table 2, demonstrated that 88 kD (U) was the product of the wheat BEIIb gene, and that while the assignment of 88 kD (L) was inconclusive, the results were consistent with the protein being a branching enzyme encoded by either SBE9 or the wheat BEIIb cDNA.

Table 2

(a) Comparison of 88 kD (U) and the predicted protein encoded by the wheat BEIIb cDNA.

Matches: 6
MOWSE Score: 4.97e+001
Coverage: 8.85%
Matching Peptides:

MW	Delta	Start	End	Sequence
755.4766	-0.13	320	325	(K) RPKSLR (I)
1337.7092	0.01	453	463	(R) VFNYGNKEVIR (F)
1337.6728	-0.03	703	713	(R) RFDLGDAEFLR (Y)
1508.7623	-0.12	785	799	(K) VVLDSDAGLFGGFGR (I)
1589.6933	-0.08	731	743	(K) YGFMTSDHQYVSR (K)
1692.7049	-0.17	184	198	(R) SDIDEHEGGMDVFSR (G)
1706.8740	-0.04	340	353	(K) INTYANFRDEVLPR (I)

(b) Comparison of 88 kD (L) and the predicted proteins encoded by the wheat BEIIb cDNA and SBE9 cDNA.

Matches to wheat BEIIb cDNA
Matches: 8
MOWSE Score: 1.32e+003
Likelihood: 2.053+003
Coverage: 11.72%
Matching Peptides:

MW	Delta	Start	End	Sequence
819.4603	11.23	464	470	(R)FLLSNAR (W)
1210.5090	-105.27	444	452	(R) GHHWMWDSR (V)
1337.7092	10.53	453	463	(R) VFNYGNKEVIR (F)
1337.6728	-16.68	703	713	(R) RFDLGDAEFLR (Y)
1508.7623	-44.33	785	799	(K) VVLDSDAGLFGGFGR (I)
1573.7446	-16.81	326	339	(R) IYETHVGMSSPEPK (I)
1589.6933	-23.46	731	743	(K) YGFMTSDHQYVSR (K)
1692.7049	-95.07	184	198	(R) SDIDEHEGGMDVFSR (G)
1706.8740	-15.57	340	353	(K) INTYANFRDEVLPR (I)

Matches to wheat SBE9
Matches: 6
MOWSE Score: 1.04e+001
Coverage: 8.63%
Matching Peptides:

MW	Delta	Start	End	Sequence
819.4603	11.23	451	457	(R)FLLSNAR (W)
1210.5090	-105.27	431	439	(R) GHHWMWDSR (V)
1508.7875	-27.64	145	156	(K) IYEIDPTLKDFR (S)
1573.7446	-16.81	313	326	(R) IYESHIGMSSPEPK (I)
1599.7641	-9.93	171	185	(R) AAIDQHEGGLEAFSR (G)
1692.8583	-4.45	327	340	(K) INSYANFRDEVLPR (I)

Example 10: *Sequencing of the SBE IIb gene*

A partial genomic sequence of the SBEIIb gene was obtained, using clone G5 described in Example 4. So far approximately 8.4kb of sequence has been obtained. This includes approximately 500bp upstream of the start codon, presumably comprising the promoter region, and exons 1 to 14 in full. This partial sequence is set out in SEQ ID NO:10. From the sequences of the corresponding maize and *Arabidopsis* BEII genes, we would expect the gene to contain 22 exons. A comparison between the exon/intron structures of various BEII genes and the wheat BEIIb gene is shown in Figure 18, and the sizes of the exons in various SBEII genes are compared in Table 3. In this table "Arab" represents *Arabidopsis*.

Table 3

Sizes of exons in various SBE IIb genes

Exon no	Arab21	Arab22	Wheat BEIIa	Maize BEIIb	Barley BEIIb	Wheat BEIIb
1	42	124	279	212	121	148
2	253	120	98	146	152	146
3	236	182	243	155	230	230
4	99	99	99	99	99	99
5	43	43	43	43	43	43
6	60	60	60	60		60
7	81	81	81	81		81
8	117	117	117	117		117
9	84	84	84	84		84
10	122	122	122	122		122
11	120	120	120	120		120
12	130	130	130	130		130
13	111	111	111	111		111
14	129	129	129	129		129
15	104	104	104	104		
16	145	145	145	145		
17		148	148	148		
18		101	101	101		
19		78	78	78		
20		156	156	156		
21		75	75	75		
22		90	384	304		
17	558					
18	164					

Using a probe specific for the 3' end of SBE IIb, three clones designated G7, G8 and G9 respectively, have

now been isolated from the *T. tauschii* genomic library, and are being subjected to sequence analysis to provide the 3' region of the gene.

Example 11: *Development of PCR Primer Sets for the Discrimination of the BEIIb Genes from each genome*

A number of primer sets, designed on the basis of comparisons between SBE IIa and SBE IIb genes, were tested on wheat genomic DNA. The sequences of these primers were as follows:

ARA 12F:	5' CCG TCC TAC ATG ACA CCT GGC CG 3'	SEQ ID NO:11
ARA 10R:	5' CCG CCG GAT CGA GGA GCC GAC GG 3'	SEQ ID NO:12
ARA 6F:	5' GGC GGC GGC GAC GGG ATG GCT GC 3'	SEQ ID NO:13
ARA 8R:	5' CGC CGT CAG GGA TCA TCA CCT CC 3'	SEQ ID NO:14
ARA 19F:	5' CAC CCA TTG TAA TTG GGT ACA CTG 3'	SEQ ID NO:15
ARA 15R	5' TCC ATG CCT CCT TCG TGT TCA TCA 3'	SEQ ID NO:16
ARA 23R	5' CTG CGC ATA AAT CCA AAC TTC TCG 3'	SEQ ID NO:17

Targeting the promoter region of SBE IIb using the primers ARA 12F and ARA 13R resulted in the specific amplification of only the D genome gene. Aneuploid analysis using this pair of primers showed that the SBE IIb was located on the long arm of chromosome 2 in wheat, as illsutrated in Figure 19.

The primers ARA6F and ARA8R, which amplify the exon 1-intron 1-exon 2 region of SBE IIb, could distinguish the D genome from the A and B genomes, as shown in Figure 20. Sequence analysis of this region indicated that the genes from the A and B genomes completely lack intron 1. This is illustrated in Figure 21.

Example 12: *Identification of SBE IIb in Genomes A, B and D*

Sequence analysis of the intron 3 region of SBE IIb, amplified by PCR using the primers ARA 19F and ARA 15R, followed by digestion using the restriction enzyme *Rsa1*,

revealed significant polymorphism amongst the three genomes. This polymorphism, illustrated in the sequnce alignment set out in Figure 22, was utilised to develop genome specific markers which can distinguish between the A, B and D genomes.

PCR amplification of the SBE IIb gene was carried out using the primers ARA 19F and ARA 15R, followed by restriction digestion using *Rsa*1. The results of the PCR analysis, shown in Figure 23, indicate that these primers can distinguish between the three genomes.

Screening of approximately 600 wheat lines using the genome specific primer pair, ARA 19F and ARA 23R, which amplifies the same region as ARA 19F and ARA 15R, identified one null mutant of the wheat genome. The amplification was performed as described for Figure 23, and the results are shown in Figure 24.

Example 13: *Constructs for Expression of BEII genes*

Recombinant DNA technology may be used to inhibit or abolish expression of either or both of BE IIa and BE IIb. Three general approaches are used, using transformation of the target plant cells with one of the following types of construct:

a) 'Antisense' constructs of SBE IIa and SBE IIb, in which the desired nucleic acid sequence is inserted into the construct in the opposite direction to the functional gene.

b) 'Sense' constructs of SBE IIa and SBE IIb, in which the desired nucleic acid is inserted in the same direction as the functional gene; this utilises co-suppression events to inhibit the expression of the target gene;

c) Duplex constructs of SBE IIa and SBE IIb, in which the desired nucleic acid in both the sense and antisense orientations is co-located in the construct on either side of a "spacer" loop formed by an intron sequence.

In all three cases, the desired nucleic acid is operably linked to a promoter sequence in the construct.

Sense and antisense constructs have been widely used to modulate gene expression in plants. More recently, it has been shown that the delivery of RNAs with potential to form duplexes is a particularly efficient strategy for generating post-transcriptional gene silencing in transgenic plants (Waterhouse et al., 1998; Smith et al., 2000).

Transformation of the target wheat cells, or cells of other plants, using these constructs is effected using methods known in the art, such as transformation with *Agrobacterium tumefaciens*. Once transgenic plants are obtained, they are assessed for the effects of the transgenes on BE IIa and BE IIb expression. For example, in both maize and potato it has been shown that crossing BE II mutations or BE II transgenes into BE I-deficient backgrounds greatly increases amylose content. Wheat BE I null lines, identified using the methods described in WO99/14314, provide a ready source of BE I-deficient genetic material into which BE IIa and BE IIb transgenics can be crossed, in order to extend further the range of starches which can be produced.

Sense, antisense and duplex constructs of SBE IIa and SBE IIb were generated in the vector pDV03000 (Biogemma Ltd, UK) which carries the high molecular weight gluten promoter (pHMWG) and the Nopaline synthase (Nos) terminator. These constructs are schematically represented in Figures 25, 26 and 27. The Biogemma vectors are based on the well-known plasmid pBR322, and comprise a number of restriction sites, as illustrated in Figures 25 and 26, for incorporation of desired DNA sequences. The entire desired DNA, plus the promoter and terminator sequences referred to above, can then be excised as a *Xho* fragment and cloned into a suitable vector, such as *Agrobacterium tumefaciens*. Those skilled in the art will be aware of other suitable vectors which could be used.

SBE IIa constructs

A sense construct of SB IIa was prepared by inserting a 2143bp fragment of SBE IIa coding sequence in the sense orientation at the *EcoR1/Sma1* site of pDV03000. An SBE IIa antisense construct was prepared by inserting 1913bp of SBE IIa coding sequence in the antisense orientation at the *EcoR1/BamH1* site of pDV03000. This is also illustrated in Figure 25.

SBE IIb constructs

A sense construct of SBE IIb was generated by inserting a 1008bp fragment of the SBE IIb coding sequence in the sense orientation at the *EcoR1/Sma1* site of pDV03000. An antisense SBE IIb construct was prepared by inserting a 955bp sequence of the coding region for SBE IIb at the *BamH1/Pst1* site of pDV03000 in the antisense orientation. This is illustrated in Figure 26.

Duplex constructs

A schematic model of a duplex construct is set out in Figure 27. The duplex construct was prepared using the following protocol, in which all the amplification steps were performed using PCR under conventional conditions.

SBE IIa duplex

1) a 468bp sequence of SBE IIa, which includes the whole of exons 1 and 2 and part of exon 3, with *EcoR1* and *Kpn1* restriction sites on either side, was amplified to obtain a first fragment (fragment 1);

2) a second fragment, 512bp in length, consisting of part of exons 3 and 4, and the whole of intron 3 of SBE IIa, with *Kpn1* and *Sac1* sites on either side, was amplified to provide fragment 2;

3) a 528bp fragment consisting of the complete exons 1, 2 and 3 of SBE IIa, with *BamH1* and *Sac1* sites on either side, was amplified to provide fragment 3;

4) fragments 1, 2 and 3 were ligated so that the sequence of fragment 3 was ligated to fragment 2 in the antisense orientation to fragment 1.

SBE IIb duplex

1) a 471bp sequence consisting of the whole of exons 1 and 2 and part of exon 3 of SBE IIb was amplified with *EcoR1* and *Kpn1* restriction sites on either side to generate fragment 1;

2) a 589bp fragment consisting of part of exons 3 and 4 and the whole of intron 3 of SBE IIb, with *Kpn1* and *Sac1* sites on either side, was amplified to provide fragment 2;

3) a 528bp fragment consisting of the complete exons 1, 2 and 3, with *BamH1* and *Sac1* sites on either side was amplified to provide fragment 3;

4) fragments 1, 2 and 3 were ligated so that fragment 3 was in the antisense orientation to fragment 1 when ligated to fragment 2.

The start and end points of the sequences used for making the constructs were as follows:

a) *SBE IIa sense construct*

Start: 461bp of Sbe 9 (SBE IIa) cDNA
End: 2603bp of Sbe 9 (SBE IIa) cDNA

b) *SBE IIa anti-sense construct*

Start: 691bp of Sbe 9 (SBE IIa) cDNA
End: 2603bp of Sbe 9 (SBE IIa) cDNA
This fragment was ligated in the anti-sense orientation.

c) *SBE IIb sense construct*

Start: 85bp of SBE IIb cDNA
End: 1085bp of SBE IIb cDNA

d) *SBE IIb anti-sense construct*

Start: 153bp of SBE IIb cDNA
End: 1085bp of SBe IIb cDNA
This fragment was ligated in the anti-sense orientation.

e) *SBE IIa duplex construct*

i) Fragment 1
Full exon 1: 1151bp - 1336bp of SBE IIa genomic sequence
Full exon 2: 1664bp - 1761bp of SBE IIa genomic sequence
Partial exon 3: 2038bp - 2219bp of SBE IIa genomic sequence
This fragment had an *EcoR1* site (GAATTC) introduced at the start of the exon 1 sequence and a *Kpn1* site (GGTACC) introduced at the end of the partial exon 3 sequence.

ii) Fragment 2
Partial exon 3: 2220bp - 2279bp of SBE IIa genomic sequence
Full intron 3: 2280bp - 2680bp of SBE IIa genomic sequence
Partial exon 4: 2681bp - 2731bp of SBE IIa genomic sequence
This fragment had a *Kpn1* site (GGTACC) introduced at the start of the partial exon 3 and a Sac1 site (GAGCTC) introduced at the end of the partial exon 4 sequence.

iii) Fragment 3
Full exon 1: 1151bp - 1336bp of SBE IIa genomic sequence
Full exon 2: 1664bp - 1761bp of SBE IIa genomic sequence
Full exon 3: 2038bp - 2279bp of SBE IIa genomic sequence

This fragment had a BamH1 site (GGATCC) introduced at the start of the complete exon 1sequence and a Sac1 site (GAGCTC) introduced at the end of the complete exon 3 sequence.

f) *SBE IIb duplex construct*

i) Fragment 1

Full exon 1: 489bp - 640bp of SBE IIb genomic sequence

Full exon 2: 789bp - 934bp of SBE IIb genomic sequence

Partial exon 3: 1598bp - 1770bp of SBE IIb genomic sequence

This fragment had an EcoR1 site (GAATTC) introduced at the start of exon 1and a Kpn1 site (GGTACC) introduced at the end of the partial exon 3 sequence.

ii) Fragment 2

Partial exon 3: 1771bp - 1827bp of SBE IIb genomic sequence

Full intron 3: 1828bp - 2292bp of SBE IIb genomic sequence

Partial exon 4: 2293bp - 2359bp of SBE IIb genomic sequence

This fragment had a Kpn1 site (GGTACC) introduced at the start of the partial exon 3 sequence and a Sac1 site (GAGCTC) introduced at the end of the partial exon 4 sequence.

iii) Fragment 3

Full exon1: 489bp - 640bp of SBE IIb genomic sequence

Full exon 2: 789bp - 934bp of SBE IIb genomic sequence

Full exon 3: 1598bp - 1827bp of SBE IIb genomic sequence

This fragment had a BamH1site (GGATCC) introduced at the start of exon 1 and a Sac1 site (GAGCTC) introduced at the end of exon 3.

The SBE IIa and SBE IIb duplexes thus formed were respectively inserted at the *Eco*R1/*Bam*H1 site of pDV03000.

Samples of λ phage clones G5 and G9 have been deposited in the Australian Government Analytical Laboratories, acting as an International Depository Authority under the Budapest Treaty on 20 February 2001, under accession numbers NM01/19255 and NM01/19256 respectively.

It will be apparent to the person skilled in the art that while the invention has been described in some detail for the purposes of clarity and understanding, various modifications and alterations to the embodiments and methods described herein may be made without departing from the scope of the inventive concept disclosed in this specification.

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CLAIMS:

1. An isolated nucleic acid molecule encoding wheat starch branching enzyme IIb (BEIIb).

2. An isolated nucleic acid molecule according to claim 1, in which the nucleic acid is a DNA.

3. An isolated nucleic acid molecule according to claim 1 or claim 2, in which the nucleic acid is a genomic DNA.

4. An isolated nucleic acid molecule according to claim 3, in which the nucleic acid is present in any one of clones G1, G2, G7 to G9, or H1 to H10.

5. An isolated nucleic acid molecule according to claim 1 or claim 2, in which the nucleic acid is a cDNA.

6. An isolated nucleic acid molecule according to claim 5, which has

(a) the sequence depicted in any one of Figure 8 (SEQ ID NO 5), Figure 9 (SEQ ID NO 6), or SEQ ID NO 10;

(b) a nucleic acid molecule capable of hybridising to at least one of the sequences in (a) under at least low stringency hybridization conditions; or

(c) a nucleic acid molecule with at least 70% sequence identity to at least one of the sequences in (a).

7. An isolated nucleic acid molecule according to claim 6, which has

(a) the sequence depicted in SEQ ID NO 10;

(b) a nucleic acid molecule capable of hybridising to SEQ ID NO:10 under at least low stringency hybridization conditions; or

(c) a nucleic acid molecu a with at least 70% sequence identity to SEQ ID NO:10.

8. An isolated nucleic acid molecule according to claim 6 or claim 7, in which the nucleic acid molecule is capable of hybridizing to at least one of the sequences in (a) under high stringency conditions, or has at least 80% sequence identity thereto.

9. An isolated nucleic acid molecule according to any one of claims 6 to 8, in which the nucleic acid molecule has at least 90% sequence identity to at least one of the sequences in (a).

10. A promoter sequence of a genomic DNA according to any one of claims 1 to 3.

11. A genetic construct comprising a nucleic acid sequence according to any one of claims 1 to 9, a biologically-active fragment thereof, or a fragment thereof encoding a biologically-active fragment of BEIIb operably linked to one or more nucleic acid sequences which are capable of facilitating expression of BEIIb in a plant.

12. A genetic construct according to claim 11, in which the plant is a cereal plant.

13. A genetic construct according to claim 11 or claim 12, in which the construct is a plasmid or a vector.

14. A genetic construct according to any one of claims 11 to 13, in which the construct is one suitable for use in transformation of a plant.

15. A genetic construct according to claim 13 or claim 14, in which the vector is a bacterium of the genus *Agrobacterium*.

16. A genetic construct according to claim 15, in which the bacterium is *Agrobacterium tumefaciens*.

17. A genetic construct for targeting of a desired gene to endosperm of a cereal plant, and/or for modulating the time of expression of a desired gene in endosperm of a cereal

plant, comprising a BEIIb promoter, operatively linked to a nucleic acid sequence encoding a desired protein, and optionally also operatively linked to one or more additional targeting sequences and/or one or more 3' untranslated sequences.

18. A genetic construct according to claim 17, in which the desired protein is encoded by a gene which is capable of being expressed in the endosperm of a cereal plant.

19. A genetic construct according to any one of claims 16 to 18, in which the desired protein is an enzyme of the starch biosynthetic pathway.

20. A genetic construct according to any one of claims 16 to 19, in which the nucleic acid encoding the desired protein is in the sense orientation.

21. A genetic construct according to claim 20, in which the sense sequence is selected from the group consisting of bacterial isoamylase, bacterial glycogen synthase, and wheat high molecular weight glutenin Bx17.

22. A genetic construct according to any one of claims 16 to 19, in which the nucleic acid encoding the desired protein is in the anti-sense orientation.

23. A genetic construct according to claim 22, in which the antisense sequence is selected from the group consisting of GBSS, starch debranching enzyme, SBE II, low molecular weight glutenin, and grain softness protein I.

24. A wheat BEIIb polypeptide, comprising an amino acid sequence encoded by a nucleic acid molecule according to any one of claims 1 to 9, or a polypeptide having at least 70% amino acid sequence identity thereto, and having the biological activity of BEIIb.

25. A wheat BEIIb polypeptide according to claim 24, having at least 80% amino acid sequence identity to an amino acid sequence encoded by a nucleic acid molecule according to any one of claims 1 to 9.

26. A wheat BEIIb polypeptide according to claim 26, having at least 90% amino acid sequence identity to an amino acid sequence encoded by a nucleic acid molecule according to any one of claims 1 to 9.

27. An antibody directed against a BEII polypeptide according to any one of claims 24 to 26.

28. An antibody according to claim 27, which is polyclonal.

29. An antibody according to claim 27, which is monoclonal.

30. An antibody according to any one of claims 27 to 29, which is raised against a sequence as set out in SEQ ID NO 7, SEQ ID NO 8, or SEQ ID NO 9.

31. A plant cell transformed by a genetic construct according to any one of claims 11 to 23.

32. A plant cell according to claim 31, which also comprises a null allele for a gene selected from the group consisting of GBSS, BEIIa, and SSII.

33. A plant derived from a cell according to claim 31 or claim 32.

34. A plant comprising one or more BEIIb null alleles, in combination with one or more other null alleles selected from the group consisting of BEIIa, GBSS, SSII and BEI, and optionally also comprising a BEIIa or BEIIb gene expressed in either the sense or the anti-sense orientation.

35. A plant according to claim 33 or claim 34, which is a cereal plant.

36. A plant according to claim 35, which is wheat or barley.

37. A product produced from a plant according to any one of claims 33 to 36.

38. A product according to claim 37, selected from the group consisting of whole grain, part grain, flour and starch.

39. A product according to claim 37 or claim 38, which is a food.

40. A food product according to claim 39, selected from the group consisting of unleavened breads, pasta, noodles, breakfast cereals, snack foods, cakes, pastries, and foods containing flour- or starch-based sauces.

41. A product according to claim 37 or claim 38, which is not a food.

42. A non-food product according to claim 41, selected from the group consisting of films, coating, adhesives, building materials, disposable goods, and packaging materials.

43. A method of modifying the characteristics of starch produced by a plant, comprising the steps of:

a) increasing the level of expression of BEIIb in the plant, or

b) decreasing the level of expression of BEIIb in the plant.

44. A method according to claim 43, in which the plant is a cereal plant.

45. A method according to claim 44, in which the plant is wheat or barley.

46. A method according to any one of claims 43 to 45, in which the branching of the amylopectin component of starch is modified.

47. A method according to any one of claims 43 to 46, in which a plant with high amylose content is produced.

48. A method according to any one of claims 43 to 46, in which a plant with high amylose content is produced.

49. A method according to any one of claims 43 to 46, in which a plant with low amylopectin content is produced.

50. A method of targeting expression of a desired gene to the endosperm of a cereal plant, comprising the step of transforming the plant with a genetic construct according to any one of claims 11 to 23.

51. A method of identifying a null or altered allele encoding an enzyme of the starch biosynthetic pathway, comprising the step of subjecting DNA from a plant suspected to possess such an allele to a DNA fingerprinting or amplification assay which utilises at least one DNA probe comprising a nucleic acid molecule according to any one of claims 1 to 10.

52. An oligonucleotide probe selected from the group consisting of SEQ ID NOS:11 to 17.

Sequence of the wheat SBE9 (BEIIa) cDNA

```
   1  ACGTTGCTCC CCCTTCTCAT CGCTTCTCAA TTAATATCTC CATCACTCGG
  51  TTCCGCGCTG CATTTCGGCC GGCGGGTTGA GTGAGATCTG GGCCACTGAC
 101  CGACTCACTC GCTCGCTGCG GGGATGGCGA CGTTCGCGGT GTCCGGCGCG
 151  ACCCTCGGTG TGGCGCGGCC GCCGGCGGCG GCGCAACCTG AAGAATTACA
 201  GATACCTGAA GACATCGAGG AGCAAACGGC TGAAGTAAAC ATGACAGGGG
 251  GGACTGCAGA AAAACTTGAA TCTTCAGAAC CGACTCAAGG CATTGTGGAA
 301  ACAATCACTG ATGGTGTAAC CAAAGGAGTT AAGGAACTAG TCGTGGGGGA
 351  GAAACCGCGA GTTGTCCCAA AACCAGGAGA TGGGCAGAAA ATATACGAGA
 401  TTGACCCAAC GCTGAAAGAT TTTCGGAGCC ATCTTGACTA CCGATACAGC
 451  GAATACAGGA GAATTCGTGC TGCTATTGAC CAACATGAAG GTGGATTGGA
 501  AGCATTTTCT CGTGGTTATG AAAAGCTTGG ATTTACCCGC AGTGCTGAAG
 551  GTATCACTTA CCGAGAATGG GCTCCTGGAG CGCATTCTGC AGCATTAGTA
 601  GGTGACTTCA ACAATTGGAA TCCGAATGCA GATACTATGA CCAGAGATGA
 651  TTATGGTGTT TGGGAGATTT TCCTCCCTAA CAATGCTGAT GGATCCCCAG
 701  CTATTCCTCA TGGCTCACGT GTAAAGATAC GGATGGATAC TCCATCTGGT
 751  GTGAAGGATT CAATTTCTGC TTGGATCAAG TTCTCTGTGC AGGCTCCAGG
 801  TGAAATACCA TTCAATGGCA TATATTATGA TCCACCTGAA GAGGAGAAGT
 851  ATGTCTTCCA ACATCCTCAA CCTAAACGAC CAGAGTCACT GAGGATTTAT
 901  GAATCACACA TTGGAATGAG CAGCCCAGAA CCGAAGATAA ATTCATATGC
 951  TAATTTTAGG GATGAGGTGC TGCCAAGAAT TAAAAGGCTT GGATACAATG
1001  CAGTGCAGAT AATGGCAATC CAGGAGCATT CATACTATGC GAGCTTTGGG
1051  TACCATGTTA CTAATTTTTT TGCACCAAGT AGCCGTTTTG GAACTCCAGA
1101  GGACTTAAAA TCCCTGATCG ATAGAGCACA TGAGCTTGGT TTGCTTGTTC
1151  TTATGGATAT TGTTCATAGT CATTCATCAA ATAATACCCT TGACGGCTTG
1201  AATGGTTTCG ATGGCACTGA TACACATTAC TTCCACGGTG GTCCACGTGG
1251  CCATCATTGG ATGTGGGATT CTCGTCTATT CAACTATGGG AGTTGGGAAG
1301  TATTGAGATT CTTACTGTCA AACGCGAGAT GGTGGCTTGA AGAATATAAG
1351  TTTGATGGAT TTCGATTTGA TGGGGTGACC TCCATGATGT ATACTCACCA
1401  TGGATTACAA ATGACATTTA CTGGGAACTA TGGCGAGTAT TTTGGATTTG
1451  CTACTGATGT TGATGCGGTA GTTTACTTGA TGCTGGTCAA CGATCTAATT
1501  CATGGACTTC ATCCTGATGC TGTATCCATT GGTGAAGATG TCAGTGGAAT
1551  GCCCACATTT TGCATCCCTG TTCCAGATGG TGGTGTTGGT TTTGACTATC
1601  GCTTGCATAT GGCTGTAGCA GATAAATGGA TTGAACTCCT CAAGCAAAGT
1651  GACGAATCTT GGAAAATGGG TGATATTGTG CACACCCTAA CAAATAGAAG
1701  GTGGCTTGAG AAGTGTGTAA CTTATGCAGA AAGTCATGAT CAAGCACTAG
1751  TTGGTGACAA GACTATTGCA TTCTGGTTGA TGGATAAGGA TATGTATGAT
1801  TTCATGGCTC TGGATAGGCC TTCAACTCCT CGCATTGATC GTGGCATAGC
1851  ATTACATAAA ATGATCAGGC TTGTCACCAT GGGTTTAGGT GGTGAAGGCT
1901  ATCTTAACTT CATGGGAAAT GAGTTTGGGC ATCCTGAATG GATAGATTTT
1951  CCAAGAGGTC CGCAAACTCT TCCAACCGGC AAAGTTCTCC CTGGAAATAA
2001  CAATAGTTAT GATAAATGCC GCCGTAGATT TGATCTTGGA GATGCAGATT
2051  TTCTTAGATA TCATGGTATG CAAGAGTTCG ATCAGGCAAT GCAGCATCTT
2101  GAGGAAAAAT ATGGGTTTAT GACATCTGAG CACCAGTATG TTTCACGGAA
2151  ACATGAGGAA GATAAGGTGA TCATCTTCGA AAGAGGAGAT TTGGTATTTG
2201  TTTTCAACTT CCACTGGAGC AATAGCTTTT TTGACTACCG TGTTGGGTGT
2251  TCCAGGCCTG GGAAGTACAA GGTGGCCTTA GACTCCGACG ATGCACTCTT
2301  TGGTGGATTC AGCAGGCTTG ATCATGATGT CGACTACTTC ACAACCGAAC
2351  ATCCGCATGA CAACAGGCCG CGCTCTTTCT CGGTGTACAC TCCGAGCAGA
2401  ACTGCGGTCG TGTATGCCCT TACAGAGTAA GAACCAGCAG CTGCTTGTTA
2451  CAAGGCAAAG AGAGAACTCC AGAGAGCTCG TGGATCGTGA GCGAAGCGAC
2501  GGGCAACGGC GCGAGGCTGC TCTAAGCGCC ATGACTGGGA GGGGATCGTG
2551  CCTCTTCCCC AGATGCCAGG AGGAGCAGAT GGATAGGTAG CTTGTTGGTG
2601  AGCGCTCGAA AGAAAATGGA CGGGCCTGGG TGTTTGTCGT GCTGCACTAC
2651  CCTCCTCCTA TCTTGCACAT TCCCGGTTGT TTTTGTACAT ATAACTAATA
2701  ATTGCCCGTG CGCTCAACGT GAACAA
```

Figure 1

Sequence of the Starch Branching Enzyme II gene (wSBE II-D1) from *A. tauschii*

```
   1  AGAAACACCT CCATTTTAGA TTTTTTTTTT GTTCTTTTCG GACGGTGGGT
  51  CGTGGAGAGA TTAGCGTCTA GTTTTCTTAA AAGAACAGGC CATTTAGGCC
 101  CTGCTTTACA AAAGGCTCAA CCAGTCCAAA ACGTCTGCTA GGATCACCAG
 151  CTGCAAAGTT AAGCGCGAGA CCACCAAAAC AGGCGCATTC GAACTGGACA
 201  GACGCTCACG CAGGAGCCCA GCACCACAGG CTTGAGCCTG ACAGCGGACG
 251  TGAGTGCGTG ACACATGGGG TCATCTATGG GCGTCGGAGC AAGGAAGAGA
 301  GACGCACATG AACACCATGA TGATGCTATC AGGCCTGATG GAGGGAGCAA
 351  CCATGCACCT TTTCCCCTCT GGAAATTCAT AGCTCACACT TTTTTTTAAT
 401  GGAAGCAAGA GTTGGCAAAC ACATGCATTT TCAAACAAGG AAAATTAATT
 451  CTCAAACCAC CATGACATGC AATTCTCAAA CCATGCACCG ACGAGTCCAT
 501  GCGAGGTGGA AACGAAGAAC TGAAAATCAA CATCCCAGTT GTCGAGTCGA
 551  GAAGAGGATG ACACTGAAAG TATGCGTATT ACGATTTCAT TTACATACAT
 601  GTACAAATAC ATAATGTACC CTACAATTTG TTTTTTGGAG CAGAGTGGTG
 651  TGGTCTTTTT TTTTTACACG AAAATGCCAT AGCTGGCCCG CATGCGTGCA
 701  GATCGGATGA TCGGTCGGAG ACGACGGACA ATCAGACACT CACCAACTGC
 751  TTTTGTCTGG GACACAATAA ATGTTTTTGT AAACAAAATA AATACTTATA
 801  AACGAGGGTA CTAGAGGCCG CTAACGGCAT GGCCAGGTAA ACGCGCTCCC
 851  AGCCGTTGGT TTGCGATCTC GTCCTCCCGC ACGCAGCGTC GCCTCCACCG
 901  TCCGTCCGTC GCTGCCACCT CTGCTGTGCG CGCGCACGAA GGGAGGAAGA
 951  ACGAACGCCG CACACACACT CACACACGGC ACACTCCCCG TGGGTCCCCT
1001  TTCCGGCTTG GCGTCTATCT CCTCTCCCCC GCCCATCCCC ATGCACTGCA
1051  CCGTACCCGC CAGCTTCCAC CCCCGCCGCA CACGTTGCTC CCCCTTCTCA
1101  TCGCTTCTCA ATTAATATCT CCATCACTCG GGTTCCGCGC TGCATTTCGG
1151  CCGGCGGGTT GAGTGAGATC TGGGCGACTG GCTGACTCAA TCACTACGCG
1201  GGGATGGCGA CGTTCGCGGT GTCCGGCGCG ACTCTCGGTG TGGCGCGGGC
1251  CGGCGTCGGA GTGGCGCGGG CCGGCTCGGA GCGGAGGGGC GGGGCGGACT
1301  TGCCGTCGCT GCTCCTCAGG AAGAAGGACT CCTCTCGTAC GCCTCGCTCT
1351  CTCGAATCTC CCCCGTCTGG CTTTGGCTCC CCTTCTCTCT CCTCTGCGCG
1401  CGCATGGCCT GTTCGATGCT GTTCCCCAAT TGATCTCCAT GAGTGAGAGA
1451  GATAGCTGGA TTAGGCGATC GCGCTTCCTG AACCTGTATT TTTTCCCCCG
1501  CGGGGAAATG CGTTAGTGTC ACCCAGGCCC TGGTGTTACC ACGGCTTTGA
1551  TCATTCCTCG TTTCATTCTG ATATATATTT TCTCATTCTT TTTCTTCCTG
1601  TTCTTGCTGT AACTGCAAGT TGTGGCGTTT TTTCACTATT GTAGTCATCC
1651  TTGCATTTTG CAGGCGCCGT CCTGAGCCGC GCGGCCTCTC CAGGGAAGGT
1701  CCTGGTGCCT GACGGCGAGA GnGACGACTT GGCAAGTCCG GCGCAACCTG
1751  AAGAATTACA GGTACACACA CTCGTGCCGG TAAATCTTCA TACAATCGTT
1801  ATTCACTTAC CAAATGCCGG ATGAAACCAA CCACGGATGC GTCAGGTTTC
1851  GAGCTTCTTC TATCAGCATT GTGCAGTACT GCACTGCCTT GTTCATTTTG
1901  TTAGCCTTGG CCCCGTGCTG GCTCTTGGGC CACTGAAAAA ATCAGATGGA
1951  TGTGCATTCT AGCAAGAACT TCACAACATA ATGCACCGTT TGGGGTTTCG
2001  TCAGTCTGCT CTACAATTGC TATTTTTCGT GCTGTAGATA CCTGAAGATA
2051  TCGAGGAGCA AACGGCGGAA GTGAACATGA CAGGGGGGAC TGCAGAGAAA
2101  CTTCAATCTT CAGAACCGAC TCAGGGCATT GTGGAAACAA TCACTGATGG
2151  TGTAACCAAA GGAGTTAAGG AACTAGTCGT GGGGGAGAAA CCGCGAGTTG
2201  TCCCAAAACC AGGAGATGGG CAGAAAATAT ACGAGATTGA CCCAACACTG
2251  AAAGATTTTC GGAGCCATCT TGACTACCGG TAATGCCTAC CCGCTGCTTT
2301  CGCTCATTTT GAATTAAGGT CCTTTCATCA TGCAAATTTG GGGAACATCA
2351  AAGAGACAAA GACTAGGGAC CACCATTTCA TACAGATCCC TTCGTGGTCT
2401  GAGAATATGC TGGGAAGTAA ATGTATAATT GATGGCTACA ATTTGCTCAA
2451  AATTGCAATA CGAATAACTG TCTCCGATCA TTACAATTAA AGAGTGGCAA
2501  ACTGATGAAA ATGTGGTGGA TGGGTTATAG ATTTTACTTT GCTAATTCCT
2551  CTACCAAATT CCTAGGGGGG AAATCTACCA GTTGGGAAAC TTAGTTTCTT
2601  ATCTTTGTGG CCTTTTTGTT TTGGGGAAAA CACATTGCTA AATTCGAATG
2651  ATTTTGGGTA TACCTCGGTG GATTCAACAG ATACAGCGAA TACAAGAGAA
2701  TTCGTGCTGC TATTGACCAA CATGAAGGTG GATTGGAAGC ATTTTCTCGT
2751  GGTTATGAAA AGCTTGGATT TACCCGCAGG TAAATTTAAA GCTTTATTAT
2801  TATGAAACGC CTCCACTAGT CTAATTGCAT ATCTTATAAG AAAATTTATA
2851  ATTCCTGTTT TCCCCTCTCT TTTTTCCAGT GCTGAAGGTA TCGTCTAATT
2901  GCATATCTTA TAAGAAAATT TATATTCCTG TTTTCCCCTA TTTTCCAGTG
2951  CTGAAGGTAT CACTTACCGA GAATGGGCTC CCTGGAGCGC ATGTTATGTT
3001  CTTTTAAGTT CCTTAACGAG ACACCTTCCA ATTTATTGTT AATGGTCACT
3051  ATTCACCAAC TAGCTTACTG GACTTACAAA TTAGCTTACT GAATACTGAC
3101  CAGTTACTAT AAATTTATGA TCTGGCTTTT GCACCCTGTT ACAGTCTGCA
3151  GCATTAGTAG GTGACTTCAA CAATTGGAAT CCAAATGCAG ATACTATGAC
3201  CAGAGTATGT CTACAGCTTG GCAATTTTCC ACCTTTGCTT CATAACTACT
3251  GATACATCTA TTTGTATTTA TTTAGCTGTT TGCACATTCC TTAAAGTTGA
```

Figure 2

```
3301  GCCTCAACTA CATCATATCA AAATGGTATA ATTTGTCAGT GTCTTAAGCT
3351  TCAGCCCAAA GATTCTACTG AATTTAGTCC ATCTTTTTGA GATTGAAAAT
3401  GAGTATATTA AGGATGAATG AATACGTGCA ACACTCCCAT CTGCATTATG
3451  TGTGCTTTTC CATCTACAAT GAGCATATTT CCATGCTATC AGTGAAGGTT
3501  TGCTCCTATT GATGCAGATA TTTGATATGG TCTTTTCAGG ATGATTATGG
3551  TGTTTGGGAG ATTTTCCTCC CTAACAACGC TGATGGATCC TCAGCTATTC
3601  CTCATGGCTC ACGTGTAAAG GTAAGCTGGC CAATTATTTA GTCGAGGATG
3651  TAGCATTTTC GAACTCTGCC TACTAAGGGT CCCTTTTCCT CTCTGTTTTT
3701  TAGATACGGA TGGATACTCC ATCCGGTGTG AAGGATTCAA TTTCTGCTTG
3751  GATCAAGTTC TCTGTGCAGG CTCCAGGTGA AATACCTTTC AATGGCATAT
3801  ATTATGATCC ACCTGAAGAG GTAAGTATCG ATCTACATTA CATTATTAAA
3851  TGAAATTTCC AGTGTTACAG TTTTTTAATA CCCACTTCTT ACTGACATGT
3901  GAGTCAAGAC AATACTTTTG AATTTGGAAG TGACATATGC ATTAATTCAC
3951  CTTCTAAGGG CTAAGGGGCA ACCAACCTTG GTGATGTGTG TATGCTTGTG
4001  TGTGACATAA GATCTTATAG CTCTTTTATG TGTTCTCTGT TGGTTAGGAT
4051  ATTCCATTTT GGCCTTTTGT GACCATTTAC TAAGGATATT TACATGCAAA
4101  TGCAGGAGAA GTATGTCTTC CAACATCTCA ACTAAACGAC CAGAGTCACT
4151  AAGGATTTAT GAATCACACA TTGGAATGAG CAGCCCGGTA TGTCAATAAG
4201  TTATTTCACC TGTTTCTGGT CTGATGGTTT ATTCTATGGA TTTTCTAGTT
4251  CTGTTATGTA CTGTTAACAT ATTACATGGT GCATTCACTT GACAACCTCG
4301  ATTTTATTTT CTAATGTCTT CATATTGGCA AGTGCAAAAC TTTGCTTCCT
4351  CTTTGTCTGC TTGTTCTTTT GTCTTCTGTA AGATTTCCAT TGCATTTGGA
4401  GGCAGTGGGC ATGTGAAAGT CATATCTATT TTTTTTTTGT CAGAGCATAG
4451  TTATATGAAT TCCATTGTTG TTGCAATAGC TCGGTATAAT GTAACCATGT
4501  TACTAGCTTA AGATTTCCCA CTTAGGATGT AAGAAATATT GCATTGGAGC
4551  GTCTCCAGCA AGCCATTTCC TACCTTATTA ATGAGAGAGA GACAAGGGGG
4601  GGGGGGGGGG GGGGGTTCCC TTCATTATTC TGCGAGCGAT TCAAAAACTT
4651  CCATTGTTCT GAGGTGTACG TACTGCAGGG ATCTCCCATT ATGAAGAGGA
4701  TATAGTTAAT TCTTTGTAAC CTACTTGGAA ACTTGAGTCT TGAGGCATCG
4751  CTAATATATA CTATCATCAC AATACTTAGA GGATGCATCT GAAnATTTTA
4801  GTGTGATCTT GCACAGGAAC CGAAGATAAA TTCATATGCT AATTTTAGGG
4851  ATGAGGTGTT GCCAAGAATT AAAAGGCTTG GATACAATGC AGTGCAGATA
4901  ATGGCAATCC AGGAGCATTC ATACTATGCA AGCTTTGGGT ATTCACACAA
4951  TCCATTTTTT TCTGTATACA CnTCTTCACC CATTTGGAGC TATTACATCC
5001  TAATGCTTCA TGCACATAAA ATATTTGGAT ATAATCCTTT ATTAGATATA
5051  TAGTACAACT ACACTTAGTA TTCTGAnnAA nAAGATCATT TTATTGTTGT
5101  TGGCTTGTTC CAGGTACCAT GTTACTAATT TTTTTGCACC AAGTAGCCGT
5151  TTTGGAACTC CAGAGGACTT AAAATCCTTG ATCGATAGAG CACATGAGCT
5201  TGGTTTGCTT GTTCTTATGG ATATTGTTCA TAGGTAATTA GTCCAATTTA
5251  ATTTTAGCTG TTTTACTGTT TATCTGGTAT TCTAAAGGGA AATTCAGGCA
5301  ATTATGATAC ATTGTCAAAA GCTAAGAGTG GCGAAAGTGA AATGTCAAAA
5351  TCTAGAGTGG CATAAGGAAA ATTGGCAAAA ACTAGAGTGG CAAAAATAAA
5401  ATTTTCCCAT CCTAAATGGC AGGGCCCTAT CGCCGAATAT TTTTCCATTC
5451  TATATAATTG TGCTACGTGA CTTCTTTTTT CTCAGATGTA TTAAACCAGT
5501  TGGACATGAA ATGTATTTGG TACATGTAGT AAACTGACAG TTCCATAGAA
5551  TATCGTTTTG TAATGGCAAC ACAATTTGAT GCCATAGATG TGGATTGAGA
5601  AGTTCAGATG CTATCAATAG AATTAATCAA CTGGCCATGT ACTCGTGGCA
5651  CTACATATAG TTTGCAAGTT GGAAAACTGA CAGCAATACC TCACTGATAA
5701  GTGGCCAGGC CCCACTTGCC AGCTTCATAC TAGATGTTAC TTCCCTGTTG
5751  AATTCATTTG AACATATTAC TTAAAGTTCT TCATTTGTCC TAAGTCAAAC
5801  TTCTTTAAGT TTGACCAAGT CTATTGGAAA ATATATCAAC ATCTACAACA
5851  CCAAATTACT TTGATCAGAT TAACAATTTT TATTTTATTA TATTAGCACA
5901  TCTTTGATGT TGTAGATATC AGCACATTTT TCTATAGACT TGGTCAAATA
5951  TAGAGAAGTT TGACTTAGGA CAAATCTAGA ACTTCAATCA ATTTGGATCA
6001  GAGGGAACAT CAAATAATAT AGATAGATGT CAACACTTCA ACAAAAAAAT
6051  CAGACCTTGT CACCATATAT GCATCAGACC ATCTGTTTGC TTTAGCCACT
6101  TGCTTTCATA TTTATGTGTT TGTACCTAAT CTACTTTTCC TTCTACTTGG
6151  TTTGGTTGAT TCTATTTCAG TTGCATTGCT TCATCAATGA TTTTGTGTAC
6201  CCTGCAGTCA TTCGTCAAAT AATACCCTTG ACGGTTTGAA TGGTTTCGAT
6251  GGCACTGATA CACATTACTT CCACGGTGGT CCACGCGGCC ATCATTGGAT
6301  GTGGGATTCT CGTCTATTCA ACTATGGGAG TTGGGAAGTA TGTAGCTCTG
6351  ACTTCTGTCA CCATATTTGG CTAACTGTTC CTGTTAATCT GTTCTTACAC
6401  ATGTTGATAT TCTATTCTTA TGCAGGTATT GAGATTCTTA CTGTCAAACG
6451  CGAGATGGTG GCTTGAAGAA TATAAGTTTG ATGGATTTCG ATTTGATGGG
6501  GTGACCTCCA TGATGTATAC TCACCATGGA TTACAAGTAA GTCATCAAGT
6551  GGTTTCAGTA ACTTTTTTAG GGCACTGAAA CAATTGCTAT GCATCATAAC
6601  ATGTATCATG ATCAGGACTT GTGCTACGGA GTCTTAGATA GTTCCCTAGT
6651  ATGCTTGTAC AATTTTACCT GATGAGATCA TGGAAGATTG GAAGTGATTA
6701  TTATTTATTT TCTTTCTAAG TTTGTTTCTT GTTCTAGATG ACATTTACTG
```

Figure 2 (cont'd)

```
 6751  GGAACTATGG CGAATATTTT GGATTTGCTA CTGATGTTGA TGCGGTAGTT
 6801  TACTTGATGC TGGTCAACGA TCTAATTCAT GGACTTTATC CTGATGCTGT
 6851  ATCCATTGGT GAAGATGTAA GTGCTTACAG TATTTATGAT TTTTAACTAG
 6901  TTAAGTAGTT TTATTTTGGG GATCAGTCTG TTACACTTTT TGTTAGGGGT
 6951  AAAATCTCTC TTTTCATAAC AATGCTAATT TATACCTTGT ATGATAATGC
 7001  ATCACTTAnG TAATTTGAAA AGTGCAAGGG CATTCAAGCT TACGAGCATA
 7051  TTTTTTGATG GCTGTAATTT ATTTGATAGT ATGCTTGTTT GGGTTTTTCA
 7101  ATAAGTGGGA GTGTGTGACT AATGTTGTAT TATTTATTTA ATTGCGGAAG
 7151  AAATGGGCAA CCTTGTCAAT TGCTTCAGAA GGCTAACTTT GATTCCATAA
 7201  ACGCTTTGGA AATGAGAGGC TATTCCCAAG GACATGAATT ATACTTCAGT
 7251  GTGTTCTGTA CATGTATTTG TAATAGTGGT TTAACTTAAA TTCCTGCACT
 7301  GCTATGGAAT CTCACTGTAT GTTGTnAGTG TACACATCCA CAAACAAGTA
 7351  ATCCTGAGCT TTCAACTCAT GAGAAAATAn GAnGTCCGCT TCTGCCAGCA
 7401  TTAACTGTTC ACAGTTCTAA TTTGTGTAAC TGTGAAATTG TTCAGGTCAG
 7451  TGGAATGCCT ACATTTTGCA TCCCTGTTCC AGATGGTGGT GTTGGTTTTG
 7501  ACTACCGCCT GCATATGGCT GTAGCAGATA AATGGATTGA ACTCCTCAAG
 7551  TAAGTGCAGG AATATTGGTG ATTACATGCG CACAATGATC TAGATTACAT
 7601  TTTCTAAATG GTAAAAAGGA AAATATGTAT GTGAATATCT AGACATTTGC
 7651  CTGTTATCAG CTTGAATACG AGAAGTCAAA TACATGATTT AAATAGCAAA
 7701  TCTCGGAAAT GTAATGGCTA GTGTCTTTAT GCTGGGCAGT GTACATTGCG
 7751  CTGTAGCAGG CCAGTCAACA CAGTTAGCAA TATTTTCAGA AACAATATTA
 7801  TTTATATCCG TATATGAnGA AAGTTAGTAT ATAAACTGTG GTCATTAATT
 7851  GTGTTCACCT TTTGTCCTGT TTAAGGATGG GCAGTAGGTA ATAAATTTAG
 7901  CCAGATAAAA TAAATCGTTA TTAGGTTTAC AAAAGGAATA TACAGGGTCA
 7951  TGTAGCATAT CTAGTTGTAA TTAATGAAAA GGCTGACAAA AGGCTCGGTA
 8001  AAAAAAACTT TATGATGATC CAGATAGATA TGCAGGAACG CGACTAAAGC
 8051  TCAAATACTT ATTGCTACTA CACAGCTGCC AATCTGTCAT GATCTGTGTT
 8101  CTGCTTTGTG CTATTTAGAT TTAAATACTA ACTCGATACA TTGGCAATAA
 8151  TAAACTTAAC TATTCAACCA ATTTGGTGGA TACCAGAnAT TTCTGCCCTC
 8201  TTGTTAGTAA TGATGTGCTC CCTGCTGCTG TTCTCTGCCG TTACAAAAGC
 8251  TGTTTTCAGT TTTTTGCATC ATTATTTTTG TGTGTGAGTA GTTTAAGCAT
 8301  GTTTTTTGAA GCTGTGAGCT GTTGGTACTT AATACATTCT TGGAAGTGTC
 8351  CAAATATGCT GCAGTGTAAT TTAGCATTTC TTTAACACAG GCAAAGTGAC
 8401  GAATCTTGGA AAATGGGCGA TATTGTGCAC ACCCTAACAA ATAGAAGGTG
 8451  GCTTGAGAAG TGTGTAACTT ATGCAGAAAG TCATGATCAA GCACTAGTTG
 8501  GTGACAAGAC TATTGCATTC TGGTTGATGG ATAAGGTACT AGCTGTTACT
 8551  TTTGGACAAA AGAATTACTC CCTCCCGTTC CTAAATATAA GTCTTTGTAG
 8601  AGATTCCACT ATGGACCACA TAGTATATAG ATGCATTTTA GAGTGTAGAT
 8651  TCACTCATTT TGCTTCGTAT GTAGTCCATA GTGAAATCTC TACAGAGACT
 8701  TATATTTAGG AACGGAGGGA GTACATAATT GATTTGTCTC ATCAGATTGC
 8751  TAGTGTTTTC TTGTGATAAA GATTGGCTGC CTCACCCATC ACCAGCTATT
 8801  TCCCAACTGT TACTTGAGCA GAATTTGCTG AAAACGTACC ATGTGGTACT
 8851  GTGGCGGCTT GTGAACTTTG ACAGTTATGT TGCAATTTTC TGTTCTTATT
 8901  TATTTGATTG CTTATGTTAC CGTTCATTTG CTCATTCCTT TCCGAGACCA
 8951  GCCAAAGTCA CGTGTTAGCT GTGTGATCTG TTATCTGAAT CTTGAGCAAA
 9001  TTTTATTAAT AGGCTAAAAT CCAACGAATT ATTTGCTTGA ATTTAAATAT
 9051  ACAGACGTAT AGTCACCTGG CTCTTTCTTA GATGATTACC ATAGTGCCTG
 9101  AAGGCTGAAA TAGTTTTGGT GTTTCTTGGA TGCCGCCTAA AGGAGTGATT
 9151  TTTATTGGAT AGATTCCTGG CCGAGTCTTC GTTACAACAT AACATTTTGG
 9201  AGATATGCTT AGTAACAGCT CTGGGAAGTT TGGTCACAAG TCTGCATCTA
 9251  CACGCTCCTT GAGGTTTTAT TATGGCGCCA TCTTTGTAAC TAGTGGCACC
 9301  TGTAAGGAAA CACATTCAAA AGGAAACGGT CACATCATTC TAATCAGGAC
 9351  CACCATACTA AGAGCAAGAT TCTGTTCCAA TTTTATGAGT TTTTGGGACT
 9401  CCAAAGGGAA CAAAAGTGTC TCATATTGTG CTTATAACTA CAGTTGTTTT
 9451  TATACCAGTG TAGTTTTATT CCAGGACAGT TGATACTTGG TACTGTGCTG
 9501  TAAATTATTT ATCCGACATA GAACAGCATG AACATATCAA GCTCTCTTTG
 9551  TGCAGGATAT GTATGATTTC ATGGCTCTGG ATAGGCTTCA ACTCTTCGCA
 9601  TTGATCGTGG CATAGCATTA CATAAAATGA TCAGGCTTGT CACCATGGGT
 9651  TTAGGTGGTG AAGGCTATCT TAACTTCATG GGAAATGAGT TTGGGCATCC
 9701  TGGTCAGTCT TTACAACATT ATTGCATTCT GCATGATTGT GATTTACTGT
 9751  AATTTGAACC ATGCTTTTCT TTCACATTGT ATGTATTATG TAATCTGTTG
 9801  CTTCCAAGGA GGAAGTTAAC TTCTATTTAC TTGGCAGAAT GGATAGATTT
 9851  TCCAAGAGGC CCACAAACTC TTCCAACCGG CAAAGTTCTc CCCTGGAAAT
 9901  AACAATAGTT ATGATAAATG CCGCCGTAGA TTTGATCTTG TAAGTTTTAG
 9951  CTGTGCTATT ACATTCCCTC ACTAGATCTT TATTGGCCAT TTATTTCTTG
10001  ATGAAATCAT AATGTTTGTT AGGAAAGATC AACATTGCTT TTGTAGTTTT
10051  GTAGACGTTA ACATAAGTAT GTGTTGAGAG TTGTTGATCA TTAAAAATAT
10101  CATGATTTTT TGCAGGGAGA TGCAGATTTT CTTAGATATC GTGGTATGCA
10151  AGAGTTCGAT CAGGCAATGC AGCATCTTGA GGAAAAATAT GGGGTATGTC
10201  ACTGGTTTGT CTTTGTTGCA TAACAAGTCA CAGTTTAACG TCAGTCTCTT
```

Figure 2 (cont'd)

```
10251   CAAGTGGTAA AAAAAGTGTA GAATTAATTC CTGTAATGAG ATGAAAACTG
10301   TGCAAAGGCG GAGCTGGAAT TGCTTTTCAC CAAAACTATT TTCTTAAGTG
10351   CTTGTGTATT GATACATATA CCAGCACTGA CAATGTAACT GCAGTTTATG
10401   ACATCTGAGC ACCAGTATGT TTCACGGAAA CATGAGGAAG ATAAGGTGAT
10451   CATCCTCnAA AAGAGGAGAT TTGGTATTTG TTTTCAACTT CCACTGGAGC
10501   AATAGCTTTT TTGACTACCG TGTTGGGTGT TCCAAGCCTG GGAAGTACAA
10551   GGTATGCTTG CCTTTTCATT GTCCACCCTT CACCAGTAGG GTTAGTGGGG
10601   GCTTCTACAA CTTTTAATTC CACATGGATA GAGTTTGTTG GTCGTGCAGC
10651   TATCAATATA AAGAATAGGG TAATTTGTAA AGAAAAGAAT TTGCTCGAGC
10701   TGTTGTAGCC ATAGGAAGGT TGTTCTTAAC AGCCCCGAAG CACATACCAT
10751   TCATTCATAT tATCTACTTA AGTGTTTGTT TCAATCTTTA TGCTCAGTTG
10801   GACTCGGTCT AATACTAGAA CTATTTTCCG AATCTACCCT AACCATCCTA
10851   GCAGTTTTAG AGCAGCCCCA TTTGGACAAT TGGCTGGGTT TTTGTTAGTT
10901   GTGACAGTTT CTGCTATTTC TTAATCAGGT GGCCTTGGAC TCTGACGATG
10951   CACTCTTTGG TGGATTCAGC AGGCTTGATC ATGATGTCGA CTACTTCACA
11001   ACCGTAAGTC TGGGCTCAAG CGTCACTTGA CTCGTCTTGA CTCAACTGCT
11051   TACAAATCTG AATCAACTTC CCAATTGCTG ATGCCCTTGC AGGAACATCC
11101   GCATGACAAC AGGCCGCGCT CTTTCTCGGT GTACACTCCG AGCAGAACTG
11151   CGGTCGTGTA TGCCCTTACA GAGTAAGAAC CAGCAGCGGC TTGTTACAAG
11201   GCAAAGAGAG AACTCCAGAG AGCTCGTGGA TCGTGAGCGA AGCGACGGGC
11251   AACGGCGCGA GGCTGCTCCA AGCGCCATGA CTGGGAGGGG ATCGTGCCTC
11301   TTCCCCAGAT GCCAGGAGGA GCAGATGGAT AGGTAGCTTG TTGGTGAGCG
11351   CTCGAAAGAA AATGGACGGG CCTGGGTGTT TGTTGTGCTG CACTGAACCC
11401   TCCTCCTATC TTGCACATTC CCGGTTGTTT TTGTACATAT AACTAATAAT
11451   TGCCCGTGCG CTCAACGTGA AAATCC
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Figure 2 (cont'd)

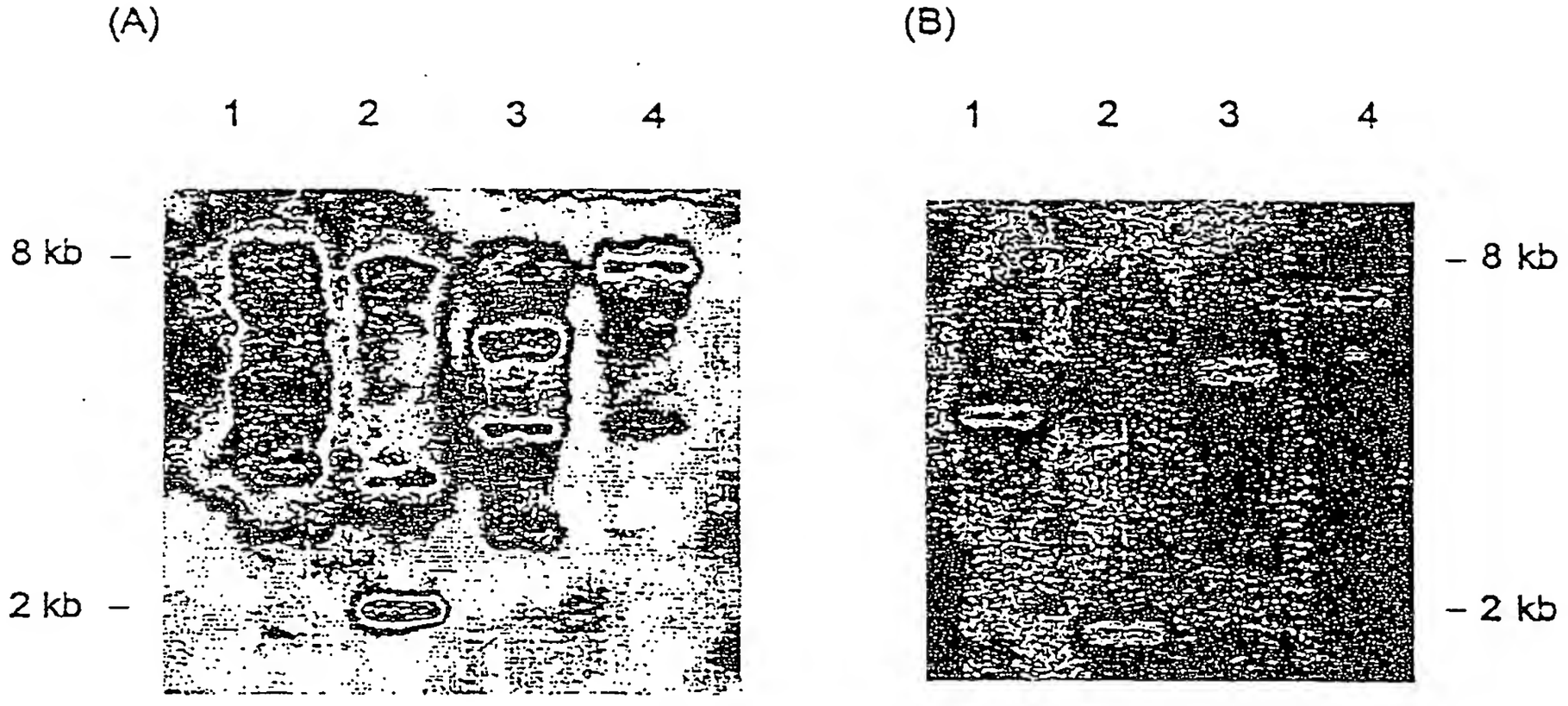


Figure 3

DNA sequence of INTRON 5 PCR Fragments

```
                1                                                    50
D genome    ATCACTTACC GAGAATGGGC TCCT.GGAGC GCATGTATGT CTTT.....
A genome    ATCACTTACC GAGAATGGGC TCCT.GGAGC GCATGTACGT CTTT.....
B genome    ATCACTTACC GAGAATGGGC TCCT.GGAGC GCATGTAC.. ..........
262bp           ATCACTTACC GAGAATGGGC TCCTGNGAGC ANATGTATGT TCTTCTGACT

               51                                                   100
D genome    ...TAAGTCT TAACAGACAC CTTCCAATTT ATTGTTAATG GT..CACTAT
A genome    ...TAAGTCT TAACAGACAC CTTCCAATTC ATTGTTAATG GTCACACTAT
B genome    ......GTCT TAACAGACAC CTTCTAATTT ATTGTTAATG GT..CACTAT
262bp       GTCTGATCGT TTACCTGACT ATACTAATTC TATCTTTCAA CTGCTTGTGA

              101                                                   150
D genome    TCACCAACTA GCTTACTGGA CTTACAAATT AGCTTACTGA ATACTGACCA
A genome    TCACCAACTA GCTTACTGGA CTTACAACTT AGCTTACTGA ATACTGACCA
B genome    TCACCAACTA GCTTACTGGA CTTACAAAAT AGCTTACTGA ATACTGACCA
262bp       ATAATTAGTG CTCATCTGCT ATCCTAAGGT TGGGGATTTT GCACTTCCCA

              151                                                   200
D genome    GTTA..... .......... ........CT ATAAATTTAT GATCTGGCTT
A genome    GTTG..... .......... ........CT CTAAATTTAT GATCTGGCTT
B genome    GTTA..... .......... ........CT CTAAATTTAT GATCTGGCTT
262bp       GATGAACAGC ATATTAAGTT GCACAACTAN CTTTATTTAA GAACTAACTC

              201                                                   250
D genome    TTGCACCCTG TTACAGTCTG CAGCATTAGT AGGTGACTTC AACAATTGGG
A genome    TTGCATCCTG TTACAGTCTG CAGCATTAGT AGGTGACTTC AACAATTGGA
B genome    TTGGATCCTG TTACAGTCTG CAGCATTAGT AGGTGACTTC AACAATTGGA
262bp       TTGCTTCCAA TTGCAGTCTG CAACATTAGT TGGCGACTTC AACAATTGGA

              251       262
D genome    ATCCAAATGC AG
A genome    ATCCAAATGC AG
B genome    ATCCAAATGC AG
262bp       ATCCAAATGC AG
```

Figure 4

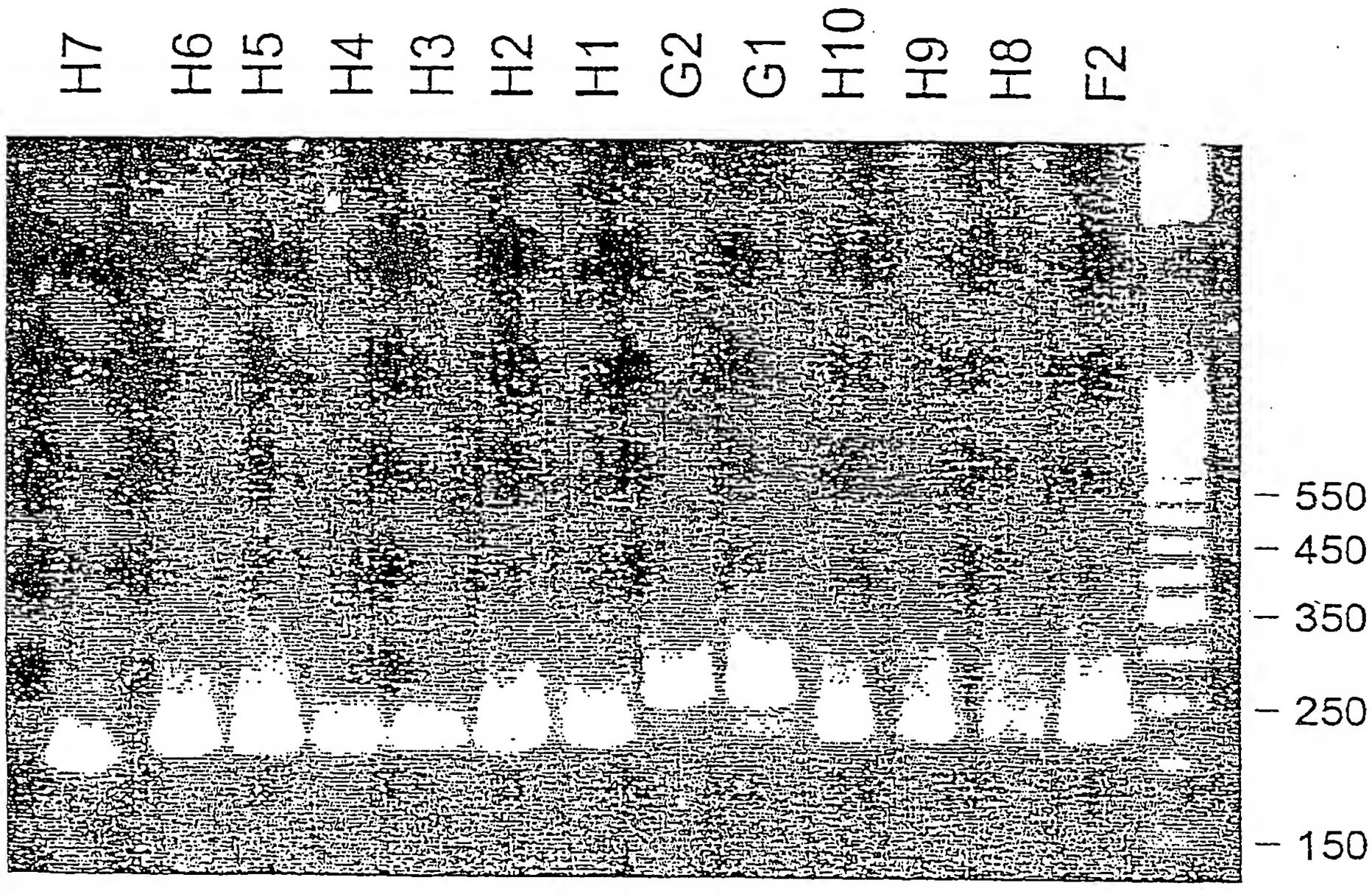


Figure 5

Comparison of Universal 262 bp Sequence with the Wheat Branching Enzyme IIb Gene

```
FILE NAME      -5          4          14         24         34         44         54

262bp           ......ATCACTTACCGAGAATGGGCTCCTGNGAGCANATGTATGTTCTTCTGACTGTCT
                      |||||||||||||||||||||||||  |||| ||||| |||||||| ||  |||
WBEIIB          GAAGGTATCACTTACCGAGAATGGGCTCCTGG-AGCAGATGTACGTTCTTCTAACCATCT
              2010       2019       2029       2039       2049       2059       2069


FILE NAME      55         64         74         84         94         104        114
262bp           GATCGTTTACCTGACTATACTAATTCTATCTTTCAACTGCTTGTGAATAATTAGTGCTCA
                |||||||||||||||||||||||||||||||||||||||||  |||||||||||||| ||||||
WBEIIB          GATCGTTTACCTGACTATACTAATTCTATCTTTCAACTAATTGTGAATAATTACTGCTCA
              2070       2079       2089       2099       2109       2119       2129


FILE NAME      115        124        134        144        154        164        174
262bp             TCTGCTATCCTAAGGTTGGGGATTTTGCACTTCCCAGATGAACAGCATATTAAGTTGCAC
                  || |||||||||||||||||||||||||| |||||||||||||||||||||||||| ||||
WBEIIB            TCAGCTATCCTAAGGTTGGGGATTTTGCACCTCCCAGATGAACAGCATATTAAGTCGCAC
              2130       2139       2149       2159       2169       2179       2189


FILE NAME      175        184        194        204        214        224        234
262bp             AACTANCTTTATTTAAGAACTAACTCTTGCTTCCAATTGCAGTCTGCAACATTAGTTGGC
                  ||||| | ||||| |||||||||||| |||||||||||||||||||| ||||||||||
WBEIIB            AACTAGCATTATT-AAGAACTAACTCCTGCTTCCAATTGCAGTCTGCAGCATTAGTTGGC
              2190       2199       2209       2219       2229       2239       2249


FILE NAME      235        244        254        264        274
262bp             GACTTCAACAATTGGAATCCAAATGCAG..............
                  ||||||||||||||| ||||||||||||
WBEIIB            GACTTCAACAATTGGGATCCAAATGCAGACCATATGAGCAAAG
              2250       2259       2269       2279       2289
```

Figure 6

Comparison of BARLEY BEIIB cDNA, WHEAT BEIIB cDNA, wheat BEIIa cDNA sequences with the wSBE II-DB1 gene

```
                          10         20         30         40         50         60         70         80         90
BARLEY BEIIB CDNA    603 TCGCAGCGCT GAAGGTATCA CTTACCGAGA ATGGGCTCCT GGAGCAGAT- ---------- ---------- ---------- ----------
692
WHEAT BEIIB CDNA CDNA     802 G********* ********** ********** ********** *********- ---------- ---------- ---------- ------
----    891
SBE9 CDNA            537 C*****T*** ********** ********** ********** *****GC**- ---------- ---------- ---------- ----------
626
WHEAT BEIIB GENE    2000 AT******** ********** ********** ********** *********G TACGTTCTTC TAACCATCTG ATCGTTTACC TGACTATACT
2089

                         100        110        120        130        140        150        160        170        180
BARLEY BEIIB CDNA    693 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
782
WHEAT BEIIB CDNA     892 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
981
SBE9 CDNA            627 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
716
WHEAT BEIIB GENE    2090 AATTCTATCT TTCAACTAAT TGTGAATAAT TACTGCTCAT CAGCTATCCT AAGGTTGGGG ATTTTGCACC TCCCAGATGA ACAGCATATT
2179

                         190        200        210        220        230        240        250        260        270
BARLEY BEIIB CDNA CDNA    783 ---------- ---------- ---------- ---------- ---------- TCTGCAGCAT TAGTTGGCGA CTTCAACAAT
TGGGATCCAA    872
WHEAT BEIIB CDNA     982 ---------- ---------- ---------- ---------- ---------- ********** ********** ********** **********
1071
SBE9 CDNA            717 ---------- ---------- ---------- ---------- ---------- ********** ****A**T** ********** ***A****G*
806
WHEAT BEIIB GENE    2180 AAGTCGCACA ACTAGCATTA TTAAGAACTA ACTCCTGCTT CCAATTGCAG ********** ********** ********** **********
2269

                         280        290        300        310        320        330        340        350        360
BARLEY BEIIB CDNA    873 CTGCAGACCA TATGAGCAAA ---------- ---------- ---------- ---------- ---------- ---------- ----------
962
WHEAT BEIIB CDNA    1072 A********* ********** ---------- ---------- ---------- ---------- ---------- ---------- ----------
1161
SBE9 CDNA            807 A*******TAC *****C**G* ---------- ---------- ---------- ---------- ---------- ---------- ----------
896
```

Figure 7

```
WHEAT BEIIB GENE   2270 A********* ********** GTATGCATGT AGTTTCACAA ATATATCATA TTTTCTTTGT AGATTTTTTT TTTTAGATCG GCTTATCT
2359

                          370        380        390        400        410        420        430        440        450
BARLEY BEIIB CDNA   963 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1052
WHEAT BEIIB CDNA   1162 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1251
SBE9 CDNA CDNA      897 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------
----   986
WHEAT BEIIB GENE   2360 TTAAATGTGG TTGAATATAC ACCTTATATG TACGTTGAGC TGTAAATATA GTTGGAAGTG TTTAGGAGTA TTAAATTCAC TGGACTCTAT
2449

                          460        470        480        490        500        510        520        530        540
BARLEY BEIIB CDNA  1053 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1142
WHEAT BEIIB CDNA   1252 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1341
SBE9 CDNA           987 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1076
WHEAT BEIIB GENE   2450 TCTTTCACTT GCCTGTTGCA CGAGCCCATT ACTAGATATC AATGTTGATG ATGCTTTTGT TGTATGAGGT CGAAGTGAAA CATGCATGTT
2539

                          550        560        570        580        590        600        610        620        630
BARLEY BEIIB CDNA  1143 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1232
WHEAT BEIIB CDNA   1342 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1431
SBE9 CDNA          1077 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1166
WHEAT BEIIB GENE   2540 ACCCTTTTAT ATAAGTAAGG TTGCACATGT ATTTTTTATG ATCTAAACAT TATTTACTGA TTTTGTTCTT GCAAGACACT AAGCAGTTTT
2629

                          640        650        660        670        680        690        700        710        720
BARLEY BEIIB CDNA  1233 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1322
WHEAT BEIIB CDNA   1432 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1521
SBE9 CDNA          1167 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1256
WHEAT BEIIB GENE   2630 ACATAATAAT GGCGTTGGAG CAGGCCGACT GCACATCTGA ACTGTAGCTC CATGTGGTTG ATATAGATTA CAAATGCTCA TATTCAATGT
2719

                          730        740        750        760        770        780        790        800        810
```

Figure 7 (cont'd)

```
BARLEY BEIIB CDNA   1323 ---------- --AATGACTT GGGTATTTGG GAGATTTTTC TGCCAAACAA TGCAGATGGT TCGCCGCCAA TTCCTCATGG CTCACGGG
1412
WHEAT BEIIB CDNA    1522 ---------- --******C* T***G***** ********** ********** ********** *****A**** *******C** **********
1611
SBE9 CDNA           1257 ---------- --G****T*A T***G***** ********C* *C**T***** ***T*****A **C**AG*T* ********** ******T**A
1346
WHEAT BEIIB GENE    2720 AACTGTTTTC AG******C* T***G***** ********** ********** ********** *****A**** *******C** **********
2809

                              820        830        840        850        860        870        880        890        900
BARLEY BEIIB CDNA   1413 AAGGT----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----GCGGAT
1502
WHEAT BEIIB CDNA    1612 *****----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----***A**
1701
SBE9 CDNA           1347 ***A*----- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----A*****
1436
WHEAT BEIIB GENE    2810 *****TGTTT TCTTCTCCTT GCCAACGGTG TTAGGCTCAG GAACATGTCC TGTATTACTC AGAAGCTCTT TTGAACATCT AGGT*A*A**
2899

                              910        920        930        940        950        960        970        980        990
BARLEY BEIIB CDNA   1503 GGATACTCCA TCTGGGACAA AGGATTCAAT TCCTGCTTGG ATCAAGTACT CCGTGCAGAC TCCAGGAGAT ATACCATACA ATGGAATATA
1592
WHEAT BEIIB CDNA    1702 **G******* ********** ********** ********** ********** ********** ********** ********** **********
1791
SBE9 CDNA           1437 ********** *****TGTG* ********** *T******** *******T** *T******G* ******T**A *******T** ****C*****
1526
WHEAT BEIIB GENE    2900 ********** *******T** ********** ********** ********** ********** ********** ********** **********
2989

                             1000       1010       1020       1030       1040       1050       1060       1070       1080
BARLEY BEIIB CDNA   1593 TTATGACCCT CCTGAAGAGG ---------- ---------- ---------- ---------- ---------- ---------- ----------
1682
WHEAT BEIIB CDNA    1792 ******T*** **C******* ---------- ---------- ---------- ---------- ---------- ---------- ----------
1881
SBE9 CDNA           1527 ******T**A ********** ---------- ---------- ---------- ---------- ---------- ---------- ----------
1616
WHEAT BEIIB GENE    2990 ******T*** **C******* TATTTTACTT CATCTTCTGT GCTTTTAGAT TTCAGATATT TTTATTAGAA GAAAATTATG ATTTTTTCCC
3079

                             1090       1100       1110       1120       1130       1140       1150       1160       1170
BARLEY BEIIB CDNA   1683 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1772
WHEAT BEIIB CDNA    1882 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
1971
```

Figure 7 (cont'd)

```
SBE9 CDNA              1617 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- --------
1706
WHEAT BEIIB GENE       3080 TCACGAACCT TCCCAATTGC TATTTCAAGC TGTCCTACTT ATTTGCTGCT GGCATCTTAT TTTTCTATTC TCTAACCAGT TATGAAATTC
3169

                                1180       1190       1200       1210       1220       1230
BARLEY BEIIB CDNA      1773 ---------- ---------AG AAGTATGTAT TCAAGCATCC TCAACCTAAA CGACCAAA.. .....
WHEAT BEIIB CDNA       1972 ---------- ---------** ********** ********** ********** *****..... .....
SBE9 CDNA              1707 ---------- ---------** ********C* **C*A***** ********** ******G*GT CACT.
WHEAT BEIIB GENE       3170 CTTACATGCA TATGCAGG** ********** ********** ********** ********AT CATTG
```

Figure 7 (cont'd)

Partial Sequence of the A. tauschii Branching Enzyme IIb gene

```
   1 GGATCCGATC CGGCTGCGGC GGCGGCGACG GGATGGCTGC GCCGGCATTC GCAGTTTCCG
  61 CGGCGGGGCT GGCCCGGCCG TCGGCTCCTC GATCCGGCGG GGCAGAGCGG AGGGGGCGCG
 121 GGGTGGAGCT GCAGTCGCCA TCGCTGCTCT TCGGCCGCAA CAAGGGCACC CGTTCACCCC
 181 GTAATTATTT GCGCCACCTT TCTCACTCAC ATTCTCTCGT GTATTCTGTC GTGCTCGCCC
 241 TTCGCCGACG ACGCGTGCCG ATTCCGTATC GGGCTGCGGT GTTCAGCGAT CTTACGTCGG
 301 TTCCCTCCTG GTGTGGTGAT GTCTGTAGGT GCCGTCGGCG TCGGAGGTTC TGGATGGCGC
 361 GTGGTCATGC GCGCGGGGGG GCCGTCCGGG GAGGTGATGA TCCCTGACGG CGGTAGTGGC
 421 GGAACACCGC CTTCCATCGA CGGTCCCGTT CAGTTCGATT CTGATGATCT GAAGGTAGTT
 481 TTTTTTTTGC ATCGATCTGA AGGTACTTGA CATATACTAC TGTATTACCC TGAGTAAATA
 541 CTGCCACCAT ATTTTTATGG TTCGCTTGAA ATACCTGTTT ACTTGCTACG GTTTTCACTT
 601 TCATTGAGAC GTCGGACGAA ATTCACTGAA TTCCTATAAT TTGGTAGACA CCGAAATATA
 661 TACTACTCCT TCCGTCCCAT AATATAAGAG CGTTTTTGGC ACCTTATATT ATAGGGCGGA
 721 GGGAGTACCT TTTAGGTCAA AATATTGTGG TAGTTTCAAT TGTATACAAG AATTCAAATA
 781 TTTTTTTTAA AAAAAAATCA ACTAATTGGT TGAGTTTCAA GTGAAGCGTT TTGGTCCTTT
 841 GGCTGAGATG TAAACCGAAA TCACTGAAAT TCATAGTAGC CGAAACTTTA ATAGAACTGA
 901 AACTCAAAAT CTGCTATCCG GCGAAATTCT AAAGATTTGC TTATTTCACA CGTAGGTTGC
 961 AGTACACCCT CTTTCTAATT TATTGGGGAA GGGGTATTAT TATCTTGTTA GTACCTGCCT
1021 GCATGACAAT TGAAATCTAA GACAAAACAC CATATGCGAG GCCTACACAC GGTAGGTTGG
1081 TTTACAACTA TGTGTGCCAC AGTTCGTCTG AACTTTTTGT CCTTCACATC GTGTTAGGTT
1141 CCATTCATTG ATGATGAAAC AAGCCTACAG GATGGAGGTG AAGATAGTAT TTGGTCTTCA
1201 GAGACAAATC AGGTTAGTGA AGAAATTGAT GCTGAAGACA CGAGCAGAAT GGACAAAGAA
1261 TCATCTACGA GGGAGAAATT ACGCATTCTG CCACCACCGG GAAATGGACA GCAAATATAC
1321 GAGATTGACC CAACGCTCCG AGACTTTAAG TACCATCTTG AGTATCGGTA TGCTTCGCTT
1381 CTATTGTGTG CACTTTAAAA ACAATTTACA GTCTTTGATA AGATGTGAAT GGCTGCTTGC
1441 TGTGACACGA AACTCTTGAA GTTCGTAGTC ACTCTTGTGT GTTCATGGTT CTGAGGTAAC
1501 ATGGTAACCG AACAAAAATA GGAAAGTGGC AAGCACTGCA ATGTGAGCTA CTGATAACCA
1561 CCCATTGTAA TTGGGTACAC TGATTAATAT ATATGTCTTC ATGGGCTCTA TTTTTTTTCA
1621 ATATCTATGC CAATTGAACA ACAATGCTTT GTGGACGGGT GTTCTTTTAC CCTCTTCTTC
1681 TATCAATAGA TGATATGCAT ACTCATGCGT ATCCTACAAA AAATTGAACA ACAATGCCAC
1741 TTTCCCCCGT GTTGCTTTTG TAAGGATGAA ACACATATGT CCAGATCAAA CTATACTAGC
1801 AGTCTAACTG TGCCTTAATG GATCAAAAAC AGATATAGCC TATACAGGAG AATACGTTCA
1861 GACATTGATG AACACGAAGG AGGCATGGAT GTATTTTCCC GCGGTTACGA GAAGTTTGGA
1921 TTTATGCGCA GGTGAAATTT CTTGACTAAA TAACTATGTA TCTACCTTTT CTTTGTACTC
1981 TATCAACATT CCTCTTCCCA TGCAGCGCTG AAGGTATCAC TTACCGAGAA TGGGCTCCTG
2041 GAGCAGATGT ACGTTCTTCT AACCATCTGA TCGTTTACCT GACTATACTA ATTCTATCTT
2101 TCAACTAATT GTGAATAATT ACTGCTCATC AGCTATCCTA AGGTTGGGGA TTTTGCACCT
2161 CCCAGATGAA CAGCATATTA AGTCGCACAA CTAGCATTAT TAAGAACTAA CTCCTGCTTC
2221 CAATTGCAGT CTGCAGCATT AGTTGGCGAC TTCAACAATT GGGATCCAAA TGCAGACCAT
2281 ATGAGCAAAG TATGCATGTA GTTTCACAAA TATATCATAT TTTCTTTGTA GATTTTTTTT
2341 TTTAGATCGG CTTATCTATT TAAATGTGGT TGAATATACA CCTTATATGT ACGTTGAGCT
2401 GTAAATATAG TTGGAAGTGT TTAGGAGTAT TAAATTCACT GGACTCTATT CTTTCACTTG
2461 CCTGTTGCAC GAGCCCATTA CTAGATATCA ATGTTGATGA TGCTTTTGTT GTATGAGGTC
2521 GAAGTGAAAC ATGCATGTTA CCCTTTTATA TAAGTAAGGT TGCACATGTA TTTTTTATGA
2581 TCTAAACATT ATTTACTGAT TTTGTTCTTG CAAGACACTA AGCAGTTTTA CATAATAATG
2641 GCGTTGGAGC AGGCCGACTG CACATCTGAA CTGTAGCTCC ATGTGGTTGA TATAGATTAC
2701 AAATGCTCAT ATTCAATGTA ACTGTTTTCA GAATGACCTT GGTGTTTGGG AGATTTTTCT
2761 GCCAAACAAT GCAGATGGTT CGCCACCAAT TCCTCACGGC TCACGGGTGA AGGTTGTTTT
2821 CTTCTCCTTG CCAACGGTGT TAGGCTCAGG AACATGTCCT GTATTACTCA GAAGCTCTTT
2881 TGAACATCTA GGTGAGAATG GATACTCCAT CTGGGATAAA GGATTCAATT CCTGCTTGGA
2941 TCAAGTACTC CGTGCAGACT CCAGGAGATA TACCATACAA TGGAATATAT TATGATCCTC
3001 CCGAAGAGGT ATTTTACTTC ATCTTCTGTG CTTTTAGATT TCAGATATTT TTATTAGAAG
3061 AAAATTATGA TTTTTTCCCT CACGAACCTT CCCAATTGCT ATTTCAAGCT GTCCTACTTA
3121 TTTGCTGCTG GCATCTTATT TTTCTATTCT CTAACCAGTT ATGAAATTCC TTACATGCAT
3181 ATGCAGGAGA AGTATGTATT CAAGCATCCT CAACCTAAAC GACCAAAATC ATTGCGGATA
3241 TATGAAACAC ATGTTGGCAT GAGTAGCCCG GTATTTCATC TTTACCATGT ATTCCATAAA
3301 TGAAGTTAGC TATATGCAGT TCAAATTTAT TTACAGGTTG TTACAATGGT ATTTTTGTGT
3361 TGGTGCCCTT CTTTCGTTTT ATAAGTAAAA AACTTATCAT AAATTTATTT GTTATGCCGC
3421 TTGGTTAATA CAATCTGAAA AATGTAACTG TGGACAATCT AGAACTAGAT AATACAAATC
3481 TGAAAAAACA TGCTGGAATA GTGTCATTTC AGTCAACTAG GATGTTTTGA ATGCTCAAGA
3541 GAAGTACTAG TGTGTAGCAT CAAAAGCTGG TGTCCATTTG TTCAAATGTT TAATTAACAC
3601 TATAGTGAAA ACAAGTAATT GCACAAAGAA ACAAGTAATT GCCCAAGTTC ATATGTTTTT
```

Figure 8

```
3661 TCACTATATT ACATGTTTCA TCAACAATTT AATTAACCTC ATTCCTTACA AACATTTGTA
3721 TTTACATTTG TTCCTACATA TATAGTTATT TTATATATCA ACTTTATAAA TCATGACTGT
3781 TATAATTAAA ACCGATGGTA TATCAACGAT TGAGATAATT TGGCATATGT GGATGAATTT
3841 TGTGGCTTGT TATGCTCTTG TTTTAATAAC ATAATAAATA GATTATGCTT GTTGGTAGCC
3901 TTTTTACATT AACACATGGG CAATTACTTG TTTCTTTGTG CAACCAGGAA CCAAAGATCG
3961 AG
//
```

Figure 8 (cont'd)

Sequence of a wheat branching enzyme IIb cDNA

```
   1  ATGGTCGACC TGCAGGCGGC CGCGAATGCA CTAGNGATTT TGACACCAGA
  51  CCAACTGGTA ATGGTAGCGA CCGGCGCTCA GCTGGAATTC GCGGCCGCGT
 101  CGACCGTGGG TTTAAGCAGG AGACGAGGCG GGGTCAGTTG GGCAGTTAGG
 151  TTGGATCCGA TCCGGCTGCG GCGGCGGCGA CGGGATGGCT GCGCCGGCAT
 201  TCGCAGTTTC CGCGGCGGGG CTGGCCCGGC CGTCGGCTCC TCGATCCGGC
 251  GGGGCAGAGC GGAGGGGGCG CGGGGTGGAG CTGCAGTCGC CATCGCTGCT
 301  CTTCGGCCGC AACAAGGGCA CCCGTTCACC CCGTGCCGTC GGCGTCGGAG
 351  GTTCTGGATG GCGCGTGGTC ATGCGCGCGG GGGGGCCGTC CGGGGAGGTG
 401  ATGATCCCTG ACGGCGGTAG TGGCGGAACA CCGCCTTCCA TCGACGGTCC
 451  CGTTCAGTTC GATTCTGATG ATCTGAAGGT TCCATTCATT GATGATGAAA
 501  CAAGCCTACA GGATGGAGGT GAAGATAGTA TTTGGTCTTC AGAGACAAAT
 551  CAGGTTAGTG AAGAAATTGA TGCTGAAGAC ACGAGCAGAA TGGACAAAGA
 601  ATCATCTACG AGGGAGAAAT TACGCATTCT GCCACCACCG GGAAATGGAC
 651  AGCAAATATA CGAGATTGAC CCAACGCTCC GAGACTTTAA GTACCATCTT
 701  GAGTATCGAT ATAGCCTATA CAGGAGAATA CGTTCAGACA TTGATGAACA
 751  CGAAGGAGGC ATGGATGTAT TTTCCCGCGG TTACGAGAAG TTTGGATTTA
 801  TGCGCAGCGC TGAAGGTATC ACTTACCGAG AATGGGCTCC TGGAGCAGAT
 851  TCTGCAGCAT TAGTTGGCGA CTTCAACAAT TGGGATCCAA ATGCAGACCA
 901  TATGAGCAAA AATGACCTTG GTGTTTGGGA GATTTTTCTG CCAAACAATG
 951  CAGATGGTTC GCCACCAATT CCTCACGGCT CACGGGTGAA GGTGCGAATG
1001  GGTACTCCAT CTGGGACAAA GGATTCAATT CCTGCTTGGA TCAAGTACTC
1051  CGTGCAGACT CCAGGAGATA TACCATACAA TGGAATATAT TATGATCCTC
1101  CCGAAGAGGA GAAGTATGTA TTCAAGCATC CTCAACCTAA ACGACCAAAA
1151  TCATTGCGGA TATATGAAAC ACATGTTGGC ATGAGTAGCC CGGAACCAAA
1201  GATCAACACA TATGCAAACT TCAGGGATGA GGTGCTTCCA AGAATTAAAA
1251  GACTTGGATA CAATGCAGTG CAAATAATGG CAATCCAAGA GCACTCATAC
1301  TATGGAAGCT TTGGGTACCA TGTTACCAAT TTCTTTGCAC CAAGTAGCCG
1351  TTTTGGGTCC CCAGAAGATT TAAAATCTTT GATTGATAGA GCTCACGAGC
1401  TTGGCTTGGT TGTCCTCATG GATGTTGTTC ACAGTCACGC GTCAAATAAT
1451  ACCTTGGACG GGTTGAATGG TTTTGATGGC ACGGATACAC ATTACTTCCA
1501  TGGCGGTTCA CGGGGCCATC ACTGGATGTG GGATTCCCGT GTGTTTAACT
1551  ATGGGAATAA GGAAGTTATA AGGTTTCTAC TTTCCAATGC AAGATGGTGG
1601  CTAGAGGAGT ATAAGTTTGA TGGTTTCCGA TTCGATGGCG CGACCTCCAT
1651  GATGTATACC CATCATGGAT TACAAGTAAC CTTTACAGGA AGCTACCATG
1701  AATATTTTGG CTTTGCCACT GATGTAGATG CGGTCGTTTA CTTGATGCTG
1751  ATGAATGATC TAATTCATGG GTTTTATCCT GAAGCCGTAA CTATCGGTGA
1801  AGATGTTAGT GGAATGCCTA CATTTGCCCT TCCTGTTCAA GTTGGTGGGG
1851  TTGGTTTTGA CTATCGCTTA CATATGGCTG TTGCCCGCAA ATGGATTGAA
1901  CTTCTCAAAG GAAACGATGA AGCTTGGGAG ATGGGTAATA TTGTGCACAC
1951  ACTAACAAAC AGAAGGTGGC TGGAAAAGTG TGTTACTTAT GCTGAAAGTC
2001  ACGATCAAGC ACTTGTTGGA GACAAGACTA TTGCATTCTG GTTGATGGAC
2051  AAGGATATGT ATGATTTCAT GGCGCTGAAC GGACCTTCGA CGCCTAATAT
2101  TGATCGTGGA ATAGCACTGC ATAAAATGAT TAGACTTATC ACAATGGGTC
2151  TAGGAGGAGA GGGTTATCTT AACTTTATGG GAAATGAGTT CGGGCATCCT
2201  GAATGGATAG ACTTTCCAAG AGGCCCACAA GTACTTCCAA GTGGTAAGTT
2251  CATCCCAGGA AACAACAACA GTTACGACAA ATGCCGTCGA AGATTTGACC
2301  TGGGTGATGC AGAATTTCTT AGGTATCATG GTATGCAGCA GTTTGATCAG
2351  GCAATGCAGC ATCTTGAGGA AAAATATGGT TTTATGACAT CAGACCACCA
2401  GTACGTATCT CGGAAACATG AGGAAGATAA GGTGATCGTG TTTGAAAAAG
2451  GGGACTTGGT ATTTGTGTTC AACTTCCACT GGAGTAGTAG CTATTTCGAC
2501  TACCGGGTCG GCTGTTTAAA GCCTGGGAAG TACAAGGTGG TCTTAGACTC
2551  GGACGCTGGA CTCTTTGGTG GATTTGGTAG GATCCATCAC ACTGCAGAGC
2601  ACTTCACTTC TGACTGCCAA CATGACAACA GGCCCCATTC ATTCTCAGTG
2651  TACACTCCTA GCAGAACCTG TGTTGTCTAT GCTCCAATGA ACTAACAGCA
2701  AAGTGCAGCA TACGCGTGCG CGCTGTTGTT GCTAGTAGCA AGAAAAATCG
2751  TATGGTCAAT ACAACCAGGT GCAAGGTTTA ATAAGGATTT TTGCTTCAAC
2801  GAGTCCTGGA TAGACAAGAC AACATGATGT TGTGCTGTGT GCTCCCAATC
2851  CCCAGGGCGT TGTGAAGAAA ACATGCTCAT CTGTGTTATT TTATGGATCA
2901  GCGACGAAAC CTCCCCCAAA TACCCCTTTT TTTTTTNAAA GGAGGATAGG
2951  CCCCCGGNCT TTGCNTNN
```

Figure 9

Alignment of Cereal Branching Enzyme Sequences

```
                1                                                    50
   Y11282       --MATFAVSG ATLGVARPAG AGGGLLPRSG SERRGGVDLP SLLLRKKDS.
     sbe9       --MATFAVSG ATLGVARPPA A......... .......... ..........
barley BEIIa    ---------- ---------- ---------- ---------- ----------
 maize BEIIa    ---------- ---------- ---------- -------DLP SVLFRRKDAF
  rice BEIV     --MASFAVSG ARLGVVRAGG GGGG..GGGP AARSGGVDLP SVLFRRKDSF
barley BEIIb    MAAPAFAV.. SAAGIARPSA R...RSSGAE PR........ .SLLFGRNKG
 wheat BEIIb    MAAPAFAV.. SAAGLARPSA P...RSGGAE RRGRGVELQS PSLLFGRNKG
 maize BEIIb    ---------- ---------- ---------- ---------- ----------
  rice BEIII    MAAPASAVPG SAAGLRAGAV RFPVPAGARS WRAAAELPTS RSLLSGR...

                51                                                   100
   Y11282       SR........ .....AVLSR AASPGKVLVP DGESDDLASP A........Q
     sbe9       .......... .......... .......... .......... A........Q
barley BEIIa    ---------- ---------- ---------- ---------- ----------
 maize BEIIa    SR........ .....TVLSC AGAPGKVLVP GGGSDDLLSS AEPVVDT..Q
  rice BEIV     SR........ .....GVVSC AGAPGKVLVP GGGSDDLLSS AEPDVETQEQ
barley BEIIb    TRFPRAVGVG GSGWRVVMRA GGPSGEVMIP DGGSGGSGTP PSIEGSVQFE
 wheat BEIIb    TRSPRAVGVG GSGWRVVMRA GGPSGEVMIP DGGSG..GTP PSIDGPVQFD
 maize BEIIb    ---------- ---------- ----KAVMVP EGENDGL... ASRADSAQFQ
  rice BEIII    .RFPGAVRVG GSGGRVAVRA AGASGEVMIP EGESDGM... PVSAG.....

                101                                                  150
   Y11282       PEELQIPEDI EEQ..... .......... ......TAEV NMTGGTAEKL
     sbe9       PEELQIPEDI EEQ..... .......... ......TAEV NMTGGTAEKL
barley BEIIa    ---------- -GE..... .......... ......MAEV NMTGGAAEKL
 maize BEIIa    PEELQIP... .......EAE LTVEKTSSSP TQTTSAVAEA SSGVEAEERP
  rice BEIV     PEESQIPDDN KVKPFEEEEE I......... ....PAVAEA SIKVVAEDKL
barley BEIIb    SDDLEVPFID D......... .......... ........EP SLHDGGEDTI
 wheat BEIIb    SDDLKVPFID D......... .......... ........ET SLQDGGEDSI
 maize BEIIb    SDELEVPDIS E......... .......... ........E. .TTCGA....
  rice BEIII    SDDLQLPALD D......... .......... ........EL STEVGAEVEI

                151                                                  200
   Y11282       ESSEPTQGIV ETITDGV... .........T KGVKELVVGE KPRVVPKPGD
     sbe9       ESSEPTQGIV ETITDGV... .........T KGVKELVVGE KPRVVPKPGD
barley BEIIa    ESSEPTQGIA ETITDGV... .........T KGVKELVVGE KPQVVPKPGD
 maize BEIIa    ELSEVI.... .....GVGGT GGTKIDGAGI K.AKAPLVEE KPRVIPPPGD
  rice BEIV     ESSEVIQDIE ENVTEGV... .........I KDADEPTVED KPRVIPPPGD
barley BEIIb    RSSETYQVTE EIDAEGVSRM D......... ...KESSTVK KIRIVPQPGN
 wheat BEIIb    WSSETNQVSE EIDAEDTSRM D......... ...KESSTRE KLRILPPPGN
 maize BEIIb    ...GVA.... ..DAQALNRV .......... .......... ..RVVPPPSD
  rice BEIII    ESSGAS.... ..DVEGVKRV V......... ...EELAAEQ KPRVVPPTGD

                201                                                  250
   Y11282       GQKIYEIDPT LKDFRSHLDY RYSEYRRIRA AIDQHEGGLE AFSRGYEKLG
     sbe9       GQKIYEIDPT LKDFRSHLDY RYSEYRRIRA AIDQHEGGLE AFSRGYEKLG
barley BEIIa    GQKIYEIDPT LKDFRSHLDY RYSEYKRIRA AIDQHEGGLE VFSRGYEKLG
 maize BEIIa    GQRIYEIDPM LEGFRGHLDY RYSEYKRLRA AIDQHEGGLD AFSRGYEKLG
  rice BEIV     GQKIYQIDPM LEGFRNHLDY RYSEYKRMRA AIDQHEGGLD AFSRGYEKLG
barley BEIIb    GQQIYDIDPM LRDFKYHLEY RYSLYRRIRS DIDEYDGGMD VFSRGYEKFG
 wheat BEIIb    GQQIYEIDPT LRDFKYHLEY RYSLYRRIRS DIDEHEGGMD VFSRGYEKFG
 maize BEIIb    GQKIFQIDPM LQGYKYHLEY RYSLYRRIRS DIDEHEGGLE AFSRSYEKFG
  rice BEIII    GQKIFQMDSM LNGYKYHLEY RYSLYRRLRS DIDQYEGGLE TFSRGYEKFG
                251                                                  300

Y11282      FTRSAEGITY REWAPGAHSA ALVGDFNNWN PNADTMTRDD YGVWEIFLPN
  sbe9      FTRSAEGITY REWAPGAHSA ALVGDFNNWN PNADTMTRDD YGVWEIFLPN
barley BEIIa  FTRSAKGITY REWAPGAHSA ALVGDFNNWN PNADTMTRDD YGVWEIFLPN
 maize BEIIa  FTRSAEGITY REWAPGAYSA ALVGDFNNWN PNADAMARNE YGVWEIFLPN
  rice BEIV   FTRSAEGITY REWAPGAQSA ALVGDFNNWN PNADTMTRNE YGVWEISLPN
barley BEIIb  FVRSAEGITY REWAPGADSA ALVGDFNNWD PTADHMSKND LGIWEIFLPN
 wheat BEIIb  FMRSAEGITY REWAPGADSA ALVGDFNNWD PNADHMSKND LGVWEIFLPN
 maize BEIIb  FNASAEGITY REWAPGAFSA ALVGDVNNWD PNADRMSKNE FGVWEIFLPN
  rice BEIII  FNHSAEGVTY REWAPGAHSA ALVGDFNNWN PNADRMSKNE FGVWEIFLPN
```

Figure 10

```
                301                                                 350
   Y11282       NADGSPAIPH GSRVKIRMDT PSGVKDSISA WIKFSVQAPG EIPFNGIYYD
     sbe9       NADGSPAIPH GSRVKIRMDT PSGVKDSISA WIKFSVQAPG EIPFNGIYYD
barley BEIIa    NADGSPAIPH GSRVKIRMDT PSGVKDSISA WIKFSVQAPG EIPFNGIYYD
 maize BEIIa    NADGSPAIPH GSRVKIRMDT PSGVKDSIPA WIKFSVQAPG EIPYNGIYYD
  rice BEIV     NADGSPAIPH GSRVKIRMDT PSGVKDSIPA WIKFAVQAPG EIPYNGIYYD
barley BEIIb    NADGSPPIPH GSRVKVRMDT PSGTKDSIPA WIKYSVQTPG DIPYNGIYYD
 wheat BEIIb    NADGSPPIPH GSRVKVRMGT PSGTKDSIPA WIKYSVQTPG DIPYNGIYYD
 maize BEIIb    NADGTSPIPH GSRVKVRMDT PSGIKDSIPA WIKYSVQAPG EIPYDGIYYD
  rice BEIII    NADGSSPIPH GSRVKVRMET PSGIKDSIPA WIKYSVQAAG EIPYNGIYYD

                351                                                 400
   Y11282       PPEEEKYVFQ HPQPKRPESL RIYESHIGMS SPEPKINSYA NFRDEVLPRI
     sbe9       PPEEEKYVFQ HPQPKRPESL RIYESHIGMS SPEPKINSYA NFRDEVLPRI
barley BEIIa    PPEEEKYVFQ HPQPKRPESL RIYESHIGMS SPEPKINSYA NFRDEVLPRI
 maize BEIIa    PPEEEKYVFK HPQPKRPKSL RIYESHVGMS SPEPKINTYA NFRDEVLPRI
  rice BEIV     PPEEEKYVFQ HPQPKRPNSL RIYESHIGMS SPEPKINTYA NFRDEVLPRI
barley BEIIb    PPEEEKYVFK HPQPKRPKSL RIYETHVGMS SPEPKINTYA NFRDEVLPRI
 wheat BEIIb    PPEEEKYVFK HPQPKRPKSL RIYETHVGMS SPEPKINTYA NFRDEVLPRI
 maize BEIIb    PPEEVKYVFR HAQPKRPKSL RIYETHVGMS SPEPKINTYV NFRDEVLPRI
  rice BEIII    PPEEEKYIFK HPQPKRPKSL RIYETHVGMS STEPKINTYA NFRDEVLPRI

                401                                                 450
   Y11282       KRLGYNAVQI MAIQEHSYYA SFGYHVTNFF APSSRFGTPE DLKSLIDRAH
     sbe9       KRLGYNAVQI MAIQEHSYYA SFGYHVTNFF APSSRFGTPE DLKSLIDRAH
barley BEIIa    KRLGYNAVQI MAIQEHSYYA SFGYHVTNFF APSSRFGTPE DLKSLIDRAH
 maize BEIIa    KKLGYNAVQI MAIQEHSYYA SFGYHVTNFF APSSRFGTPE DLKSLIDKAH
  rice BEIV     KKLGYNAVQI MAIQEHSYYA SFGYHVTNFF APSSRFGTPE DLKSLIDKAH
barley BEIIb    KRLGYNAVQI MAIQEHSYYG SFGYHVTNFF APSSRFGSPE DLKSLIDRAH
 wheat BEIIb    KRLGYNAVQI MAIQEHSYYG SFGYHVTNFF APSSRFGSPE DLKSLIDRAH
 maize BEIIb    KKLGYNAVQI MAIQEHSYYG SFGYHVTNFF APSSRFGTPE DLKSLIDRAH
  rice BEIII    KKLGYNAVQI MAIQEHAYYG SFGYHVTNFF APSSRFGTPE DLKSLIDKAH

                451                                                 500
   Y11282       ELGLLVLMDI VHSHSSNNTL DGLNGFDGTD THYFHGGPRG HHWMWDSRLF
     sbe9       ELGLLVLMDI VHSHSSNNTL DGLNGFDGTD THYFHGGPRG HHWMWDSRLF
barley BEIIa    ELGLLVLMDI VHSHSSNNTL DGLNGFDGTD THYFHGGPRG HHWMWDSRLF
 maize BEIIa    ELGLLVLMDI VHSHSSNNTL DGLNGFDGTD THYFHGGPRG HHWMWDSRLF
  rice BEIV     ELGLLVLMDI VHSHASNNTL DGLNGFDGTD THYFHGGPRG HHWMWDSRLF
barley BEIIb    ELGLLVLMDV VHSHASSNTL DGLNGFDGTD THYFHGGSRG HHWMWDSRVF
 wheat BEIIb    ELGLVVLMDV VHSHASNNTL DGLNGFDGTD THYFHGGSRG HHWMWDSRVF
 maize BEIIb    ELGLLVLMDV VHSHASSNTL DGLNGFDGTD THYFHSGPRG HHWMWDSRLF
  rice BEIII    ELGLVVLMDV VHSHASNNTL DGLNGFDGTD THYFHSGSRG HHWMWDSRLF

                501                                                 550
   Y11282       NYGSWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQMTFTGNY
     sbe9       NYGSWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQMTFTGNY
barley BEIIa    NYGSWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQMTFTGNY
 maize BEIIa    NYGSWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQVTFTGNY
  rice BEIV     NYGSWEVLRY LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQVAFTGNY
barley BEIIb    NYGNKEVIRF LLSNARWWLE EYKFDGFRFD GATSMMYTHH GLQVTFTGSY
 wheat BEIIb    NYGNKEVIRF LLSNARWWLE EYKFDGFRFD GATSMMYTHH GLQVTFTGSY
 maize BEIIb    NYGNWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQVTFTGNF
  rice BEIII    NYGNWEVLRF LLSNARWWLE EYKFDGFRFD GVTSMMYTHH GLQVAFTGNY

                551                                                 600
   Y11282       GEYFGFATDV DAVVYLMLVN DLIHGLHPDA VSIGEDVSGM PTFCIPVPDG
     sbe9       GEYFGFATDV DAVVYLMLVN DLIHGLHPDA VSIGEDVSGM PTFCIPVPDG
barley BEIIa    GEYFGFATDV DAVVYLMLVN DLIHGLYPDA VSIGEDVSGM PTFCIPVPDG
 maize BEIIa    GEYFGFATDV DAVVYLMLVN DLIRGLYPEA VSIGEDVSGM PTFCIPVQDG
  rice BEIV     GEYFGFATDV DAVVYLMLVN DLIHGLYPEA VAIGEDVSGM PTFCIPVQDG
barley BEIIb    HEYFGFATDV DAVVYLMLVN DLIHALYPEA VTIGEDVSGM PTFALPVQVG
 wheat BEIIb    HEYFGFATDV DAVVYLMLMN DLIHGFYPEA VTIGEDVSGM PTFALPVQVG
 maize BEIIb    NEYFGFATDV DAVVYLMLVN DLIHGLYPEA VTIGEDVSGM PTFALPVHDG
  rice BEIII    SEYFGFATDA DAVVYLMLVN DLIHGLYPEA ITIGEDVSGM PTFALPVQDG
```

Figure 10 (cont'd)

```
                601                                                  650
   Y11282       GVGLDYRLHM AVADKWIELL KQSDESWKMG DIVHTLTNRR WLEKCVTYAE
     sbe9       GVGFDYRLHM AVADKWIELL KQSDESWKMG DIVHTLTNRR WLEKCVTYAE
barley BEIIa    GVGFDYRLHM AVADKWIELL KQSDESWKMG DIVHTLTNRR WLEKCVTYAE
 maize BEIIa    GVGFDYRLHM AVPDKWIELL KQSDEYWEMG DIVHTLTNRR WLEKCVTYCE
  rice BEIV     GVGFDYRLHM AVPDKWIELL KQSDEYWKMG DIVHTLTNRR WSEKCVTYAE
barley BEIIb    GVGFDYRLHM AVADKWIELL KGSDEGWEMG NIVHTLTNRR WLEKCVTYAE
 wheat BEIIb    GVGFDYRLHM AVARKWIELL KGNDEAWEMG NIVHTLTNRR WLEKCVTYAE
 maize BEIIb    GVGFDYRMHM AVADKWIDLL KQSDETWKMG DIVHTLTNRR WLEKCVTYAE
  rice BEIII    GVGFDYRLHM AVPDKWIELL KQSDESWKMG DIVHTLTNRR WSEKCVTYAE

                651                                                  700
   Y11282       SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPRIDRGIA LHKMIRLVTM
     sbe9       SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPRIDRGIA LHKMIRLVTM
barley BEIIa    SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPRIDRGIA LHKMIRLVTM
 maize BEIIa    SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPRIDRGIA LHKMIRLVTM
  rice BEIV     SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPRIDRGIA LHKMIRLVTM
barley BEIIb    SHDQALVGDK TIAFWLMDKD MYDFMALNGP STPNIDRGIA LHKMIRLITM
 wheat BEIIb    SHDQALVGDK TIAFWLMDKD MYDFMALNGP STPNIDRGIA LHKMIRLITM
 maize BEIIb    SHDQALVGDK TIAFWLMDKD MYDFMALDRP STPTIDRGIA LHKMIRLITM
  rice BEIII    SHDQALVGDK TIAFWLMDKD MYDFMALDRP ATPSIDRGIA LHKMIRLITM

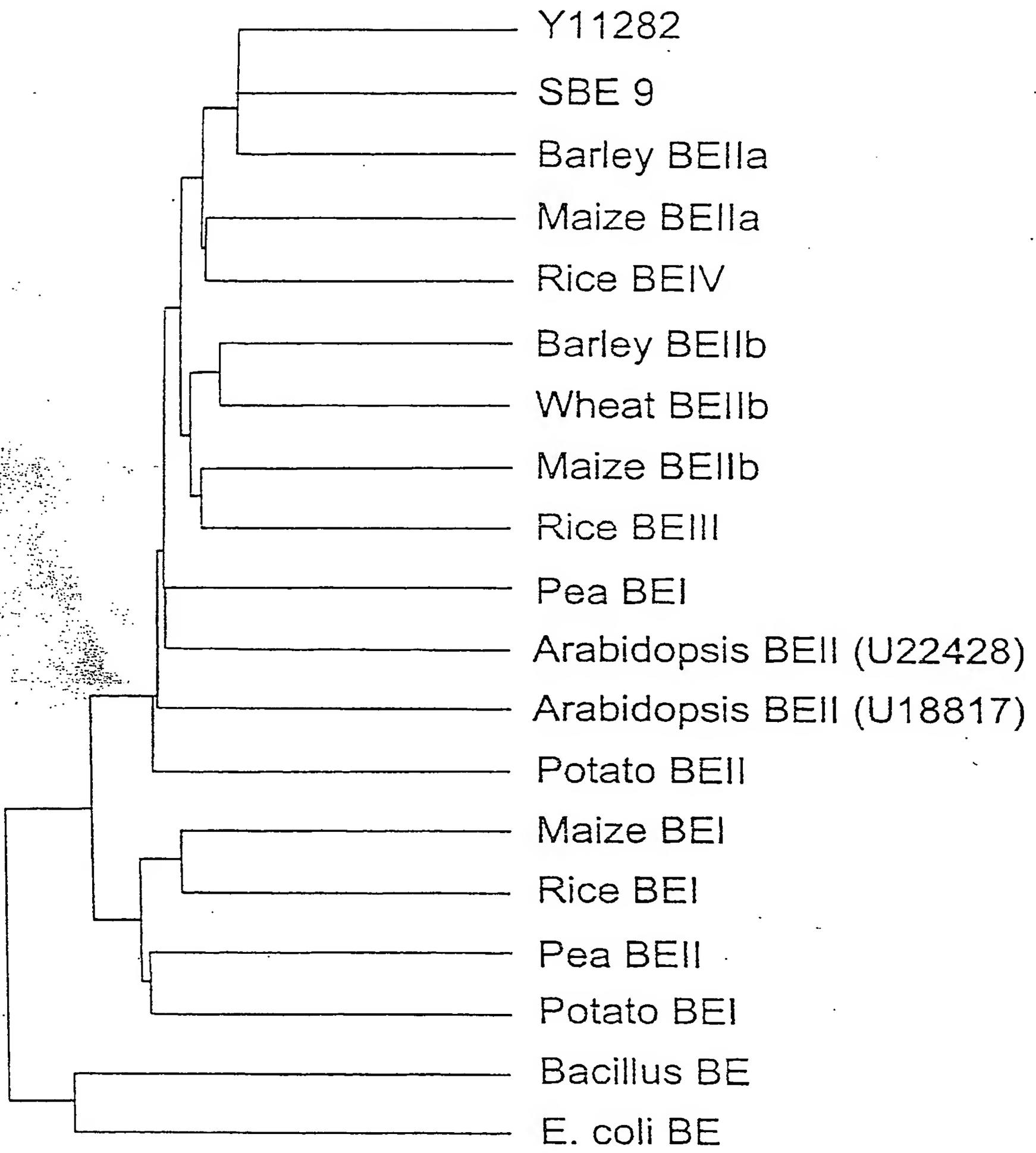
                701                                                  750
   Y11282       GLGGEGYLNF MGNEFGHPEW IDFPRGPQTL PTGKVLPGNN NSYDKCRRRF
     sbe9       GLGGEGYLNF MGNEFGHPEW IDFPRGPQTL PTGKVLPGNN NSYDKCRRRF
barley BEIIa    GLGGEGYLNF MGNEFGHPEW IDFPRGPQTL PTGKVLPGNN NSYDKCRRRF
 maize BEIIa    GLGGEGYLNF MGNEFGHPEW IDFPRGPQSL PNGSVIPGNN NSFDKCRRRF
  rice BEIV     GLGGEGYLNF MGNEFGHPEW IDFPRGPQSL PNGSVLPGNN YSFDKCRRRF
barley BEIIb    ALGGEGYLNF MGNEFGHPEW IDFPRGPQVL PTGKFIPGNN NSYDKCRRRF
 wheat BEIIb    GLGGEGYLNF MGNEFGHPEW IDFPRGPQVL PSGKFIPGNN NSYDKCRRRF
 maize BEIIb    GLGGEGYLNF MGNEFGHPEW IDFPRGPQRL PSGKFIPGNN NSYDKCRRRF
  rice BEIII    GLGGEGYLNF MGNEFGHPEW IDFPRAPQVL PNGKFIPGNN NSYDKCRRRF

                751                                                  800
   Y11282       DLGDADFLRY HGMQEFDQAM QHLEEKYGFM TSEHQYVSRK HEEDKVIIFE
     sbe9       DLGDADFLRY HGMQEFDQAM QHLEEKYGFM TSEHQYVSRK HEEDKVIIFE
barley BEIIa    DLGDADFLRY RGMQEFDQAM QHLEEKYGFM TSEHQYVSRK HEEDKVIIFE
 maize BEIIa    DLGDADYLRY RGMQEFDQAM QHLEGKYEFM TSDHSYVSRK HEEDKVIIFE
  rice BEIV     DLGDADYLRY HGMQEFDQAM QHLEEKYGFM TSEHQYISRK HEEDKVIIFE
barley BEIIb    DLGDAEFLRY HGMQQFDQAM QHLEEKYGFM TSDHQYVSRK HEEDKVIVFE
 wheat BEIIb    DLGDAEFLRY HGMQQFDQAM QHLEEKYGFM TSDHQYVSRK HEEDKVIVFE
 maize BEIIb    DLGDADYLRY HGMQEFDQAM QHLEQKYEFM TSDHQYISRK HEEDKVIVFE
  rice BEIII    DLGDADYLRY RGMLEFDRAM QSLEEKYGFM TSDHQYISRK HEEDKMIIFE

                801                                                  850
   Y11282       RGDLVFVFNF HWSNSFFDYR VGCSRPGKYK VALDSDDALF GGFSRLDHDV
     sbe9       RGDLVFVFNF HWSNSFFDYR VGCSRPGKYK VALDSDDALF GGFSRLDHDV
barley BEIIa    RGDLVFVFNF HWSNSKKDYR VGCSKPGKYK VALDSDDALF GGFSRLDHDV
 maize BEIIa    RGDLVFVFNF HWSNSYFDYR VGCFKPGKYK IVLDSDDGLF GGFSRLDHDA
  rice BEIV     RGDLVFVFNF HWSNSYFDYR VGCLKPGKYK IVLDSDDGLF GGFSRLDHDA
barley BEIIb    KGDLVFVFNF HWSNSYFDYR VGCLKPGKYK VVLDSDAGLF GGFGRIHHTG
 wheat BEIIb    KGDLVFVFNF HWSSSYFDYR VGCLKPGKYK VVLDSDAGLF GGFGRIHHTA
 maize BEIIb    KGDLVFVFNF HCNNSYFDYR IGCRKPGVYK VVLDSDAGLF GGFSRIHHAA
  rice BEIII    KGDLVFVFNF HWSNSYFDYR VGCLKPGKYK VVLDSDAGLF GGFGRIHHTA

                851                                                  887
   Y11282       DYFTTEHPHD NRPRSFSVYT PSRTAVVYAL TE*-----
     sbe9       DYFTTEHPHD NRPRSFSVYT PSRTAVVYAL TE*-----
barley BEIIa    DYFTTEHPHD NRPRSFSVYT PSRTAVVYAL TE*-----
 maize BEIIa    EYFTADWPHD NRPCSFSVYA PSRTAVVYAP AGAEDE*
  rice BEIV     EYFTADWPHD NRPCSFSVYT PSRTAVVYAL ..TED*-
barley BEIIb    EHFTNGCQHD NRPHSFSVYT PSRTCVVYAP MN*-----
 wheat BEIIb    EHFTSDCQHD NRPHSFSVYT PSRTCVVYAP MN*-----
 maize BEIIb    EHFTADCSHD NRPYSFSVYT PSRTCVVYAP VE*-----
  rice BEIII    EHFTADCSHD NRPYSFSVYS PSRTCVVYAP AE*-----
```

Figure 10 (cont'd)


Y11282
SBE 9
Barley BEIIa
Maize BEIIa
Rice BEIV
Barley BEIIb
Wheat BEIIb
Maize BEIIb
Rice BEIII
Pea BEI
Arabidopsis BEII (U22428)
Arabidopsis BEII (U18817)
Potato BEII
Maize BEI
Rice BEI
Pea BEII
Potato BEI
Bacillus BE
E. coli BE

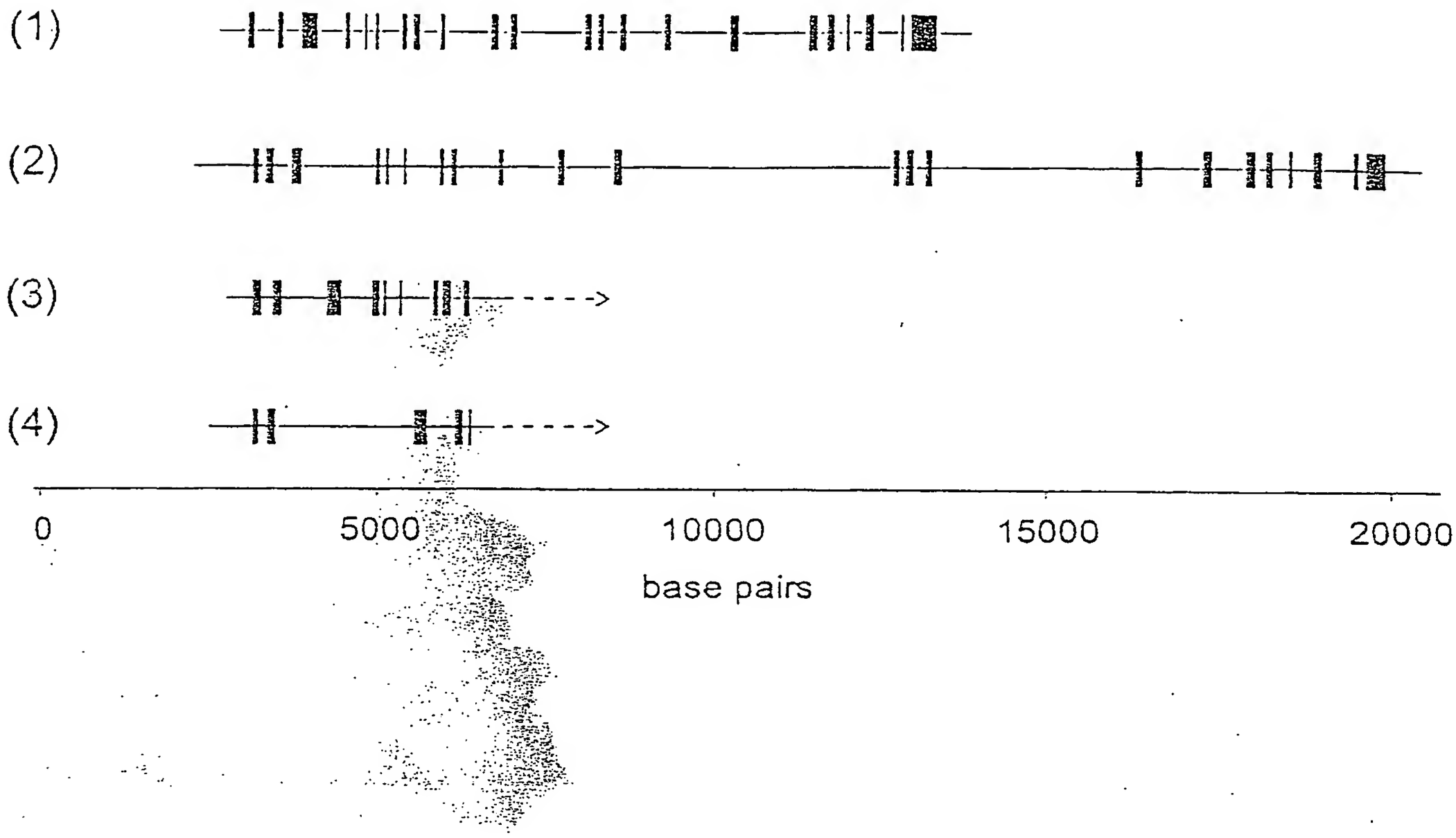


Figure 12

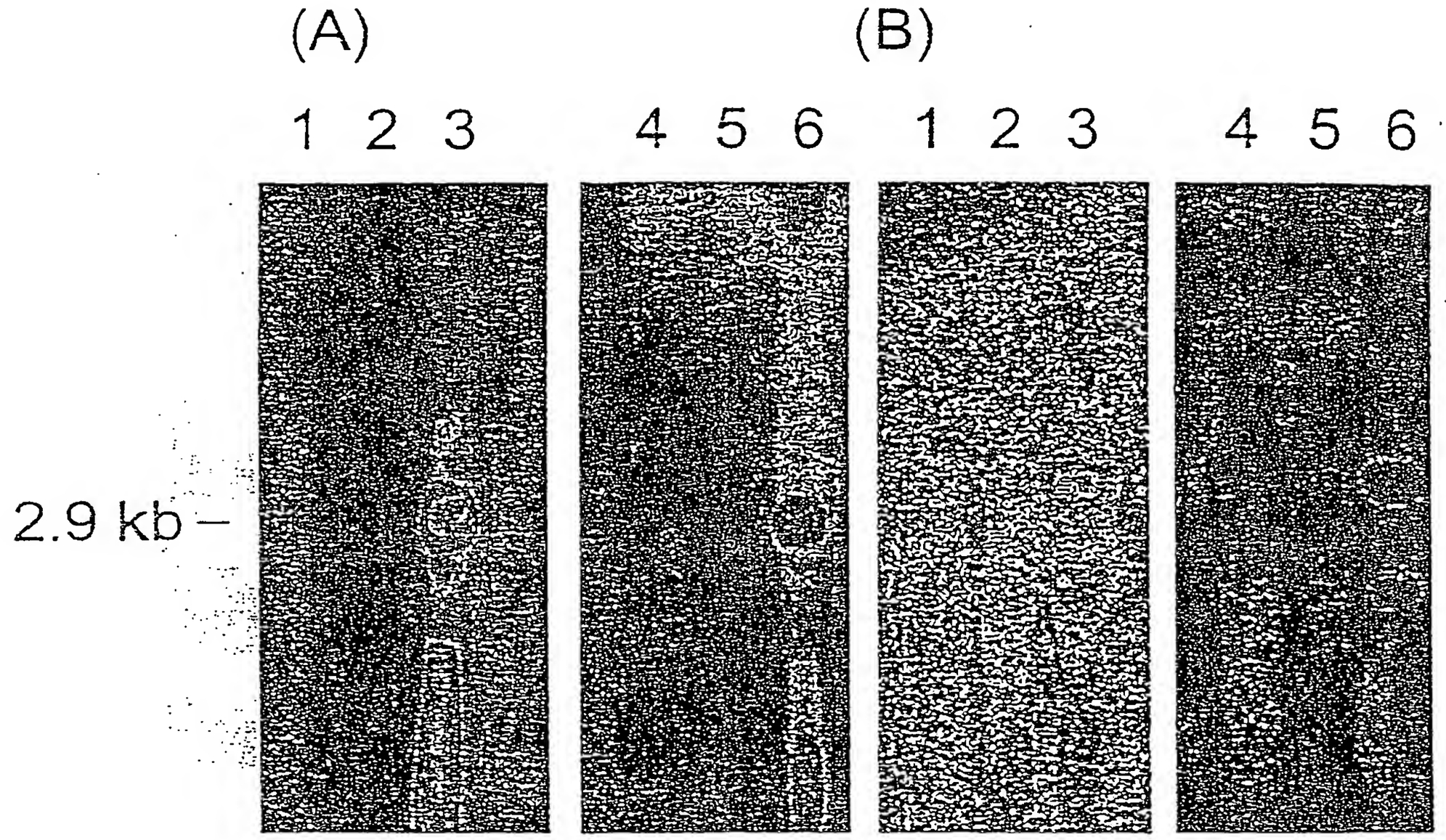


Figure 13

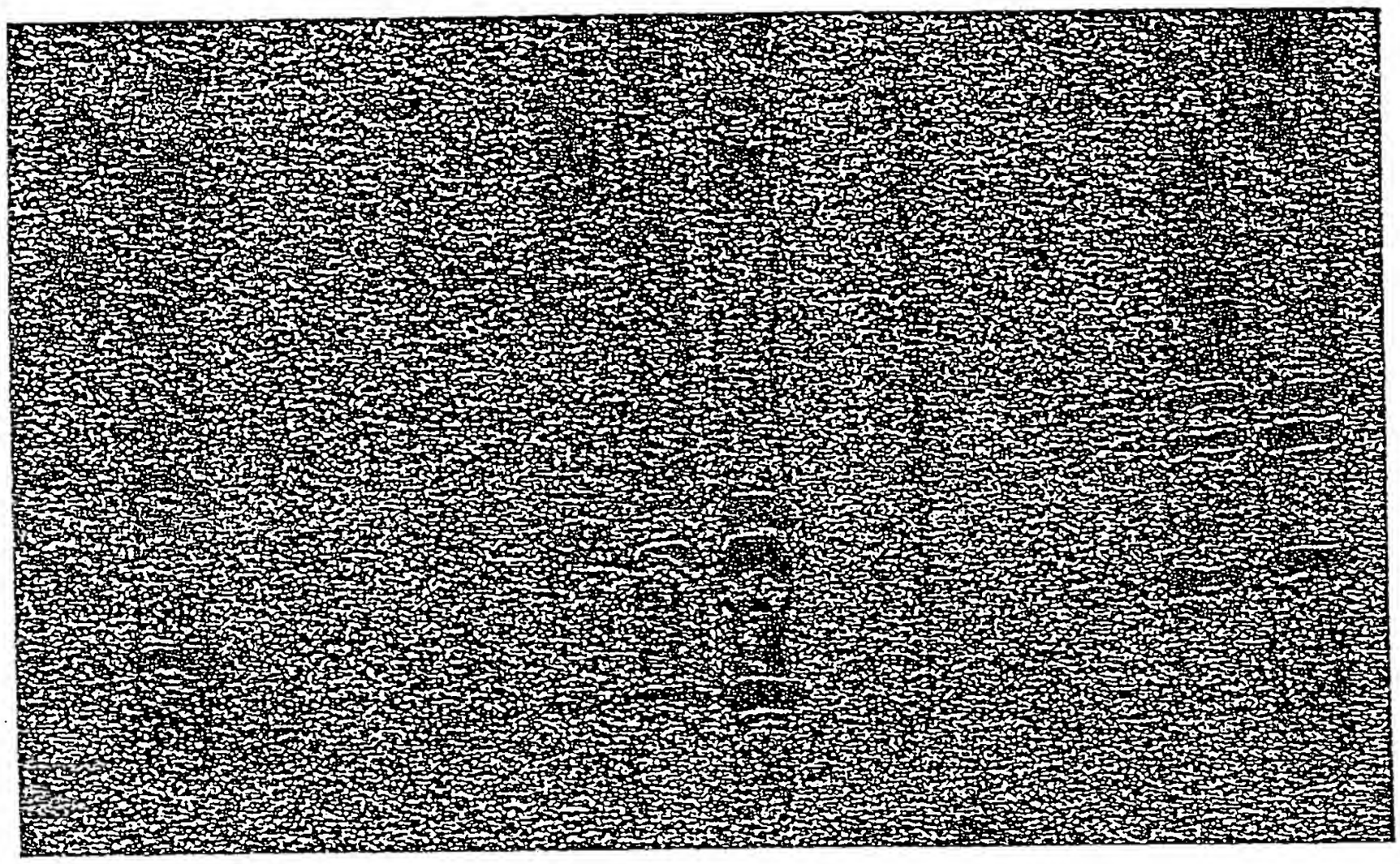


Figure 14

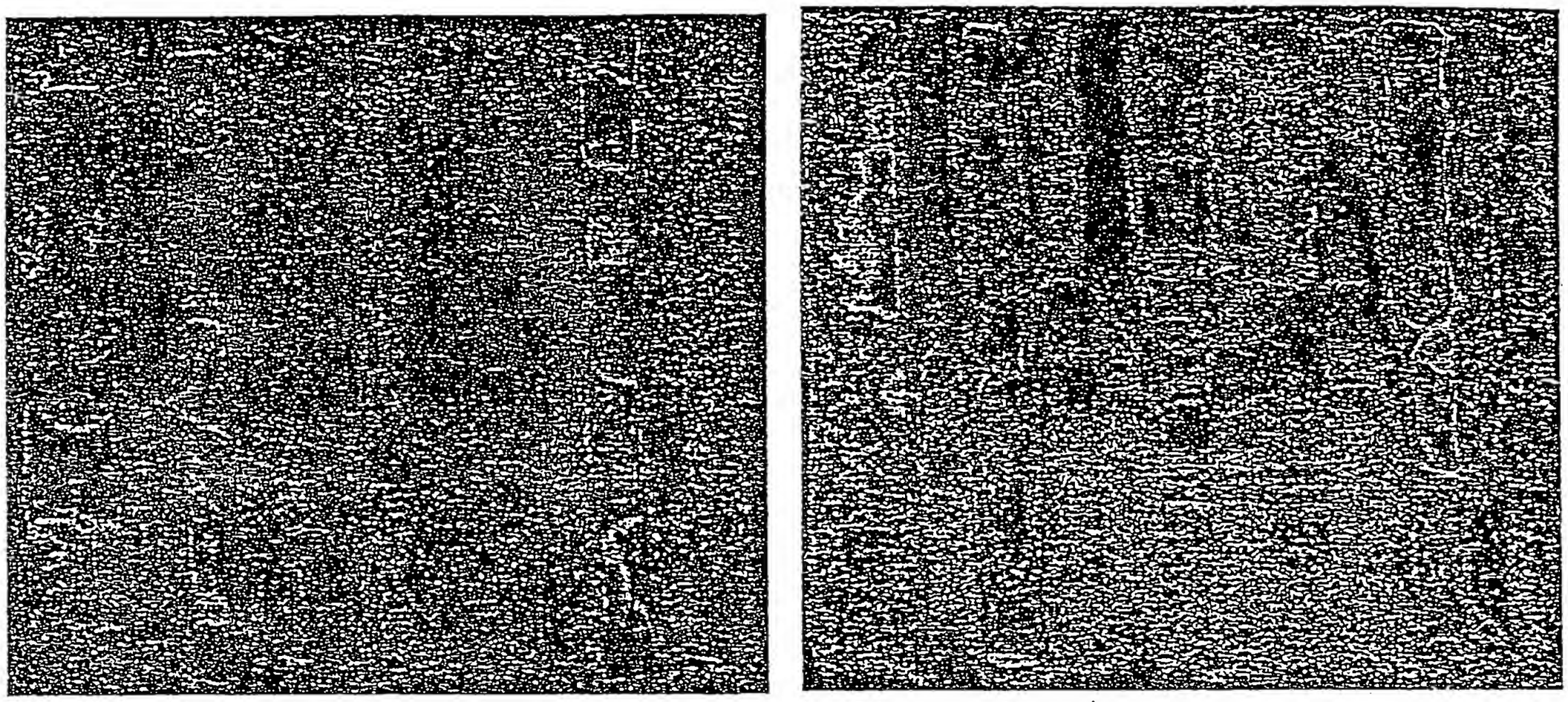
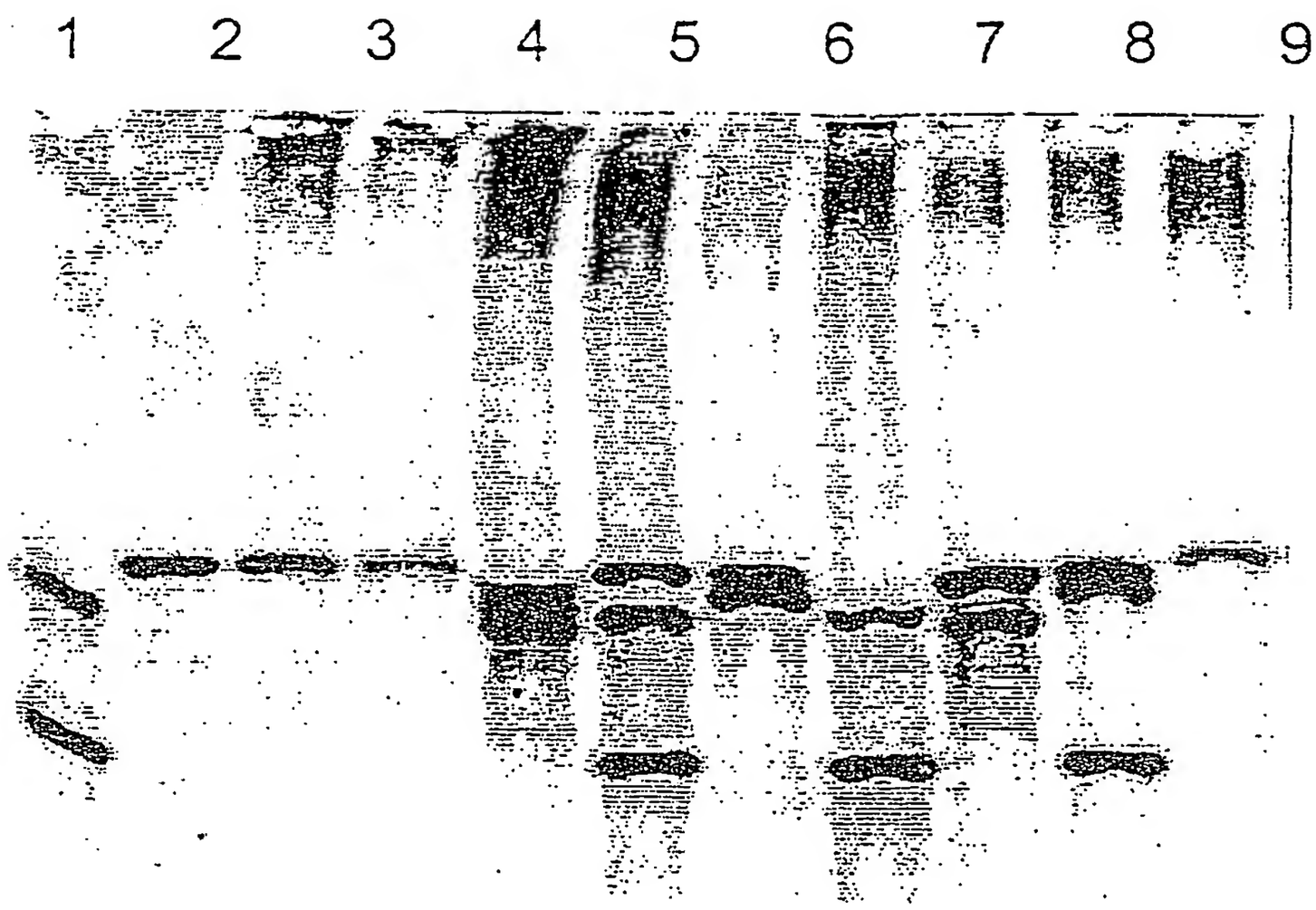


Figure 15

(A)



(B)

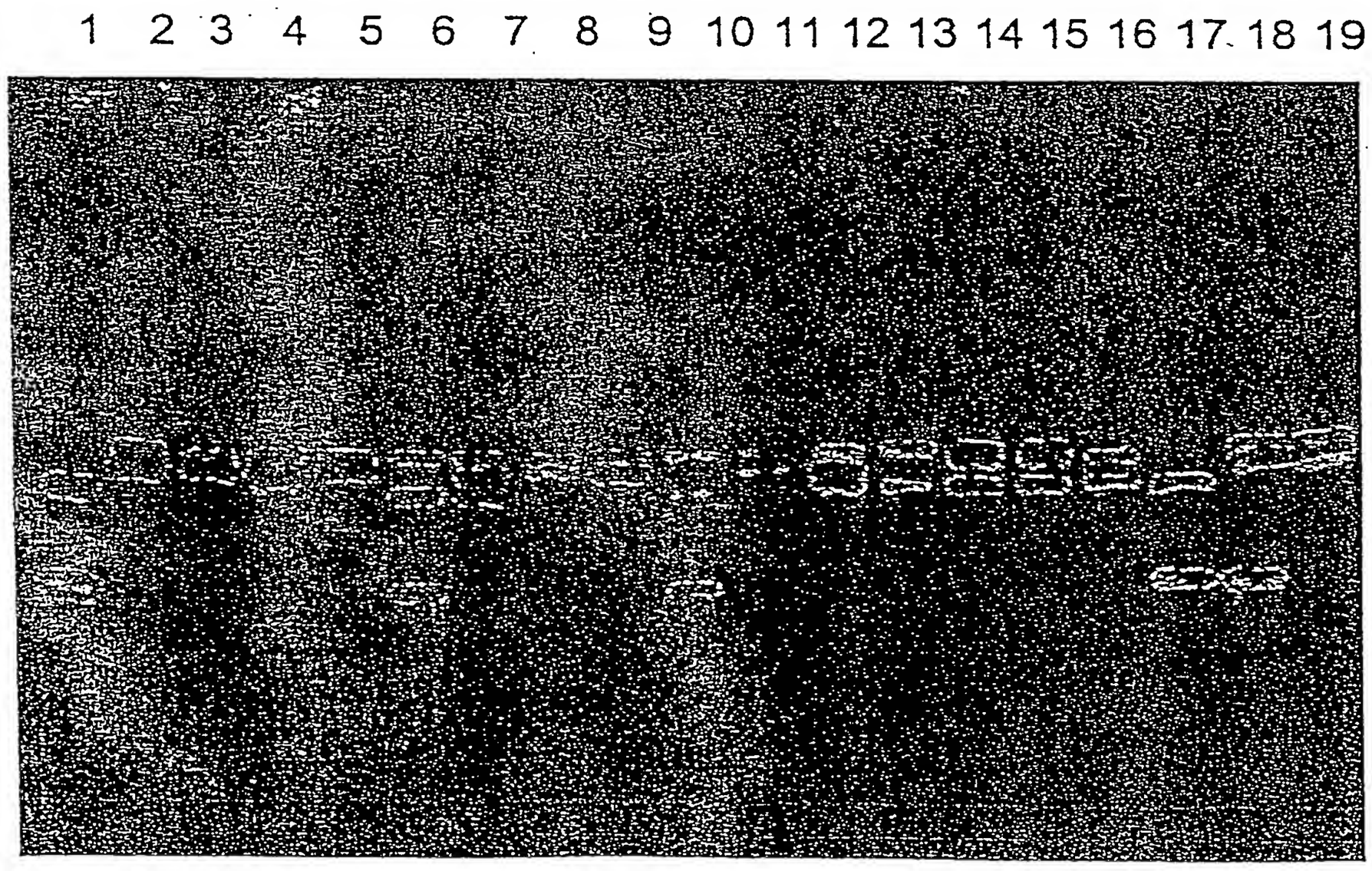
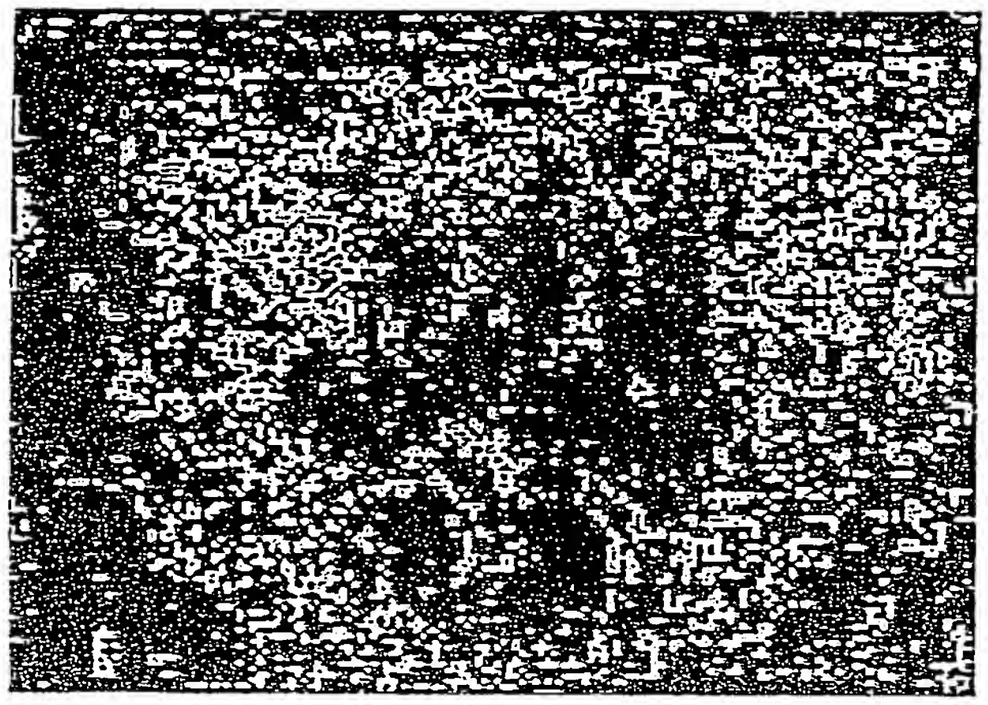


Figure 16

(A)



(B)

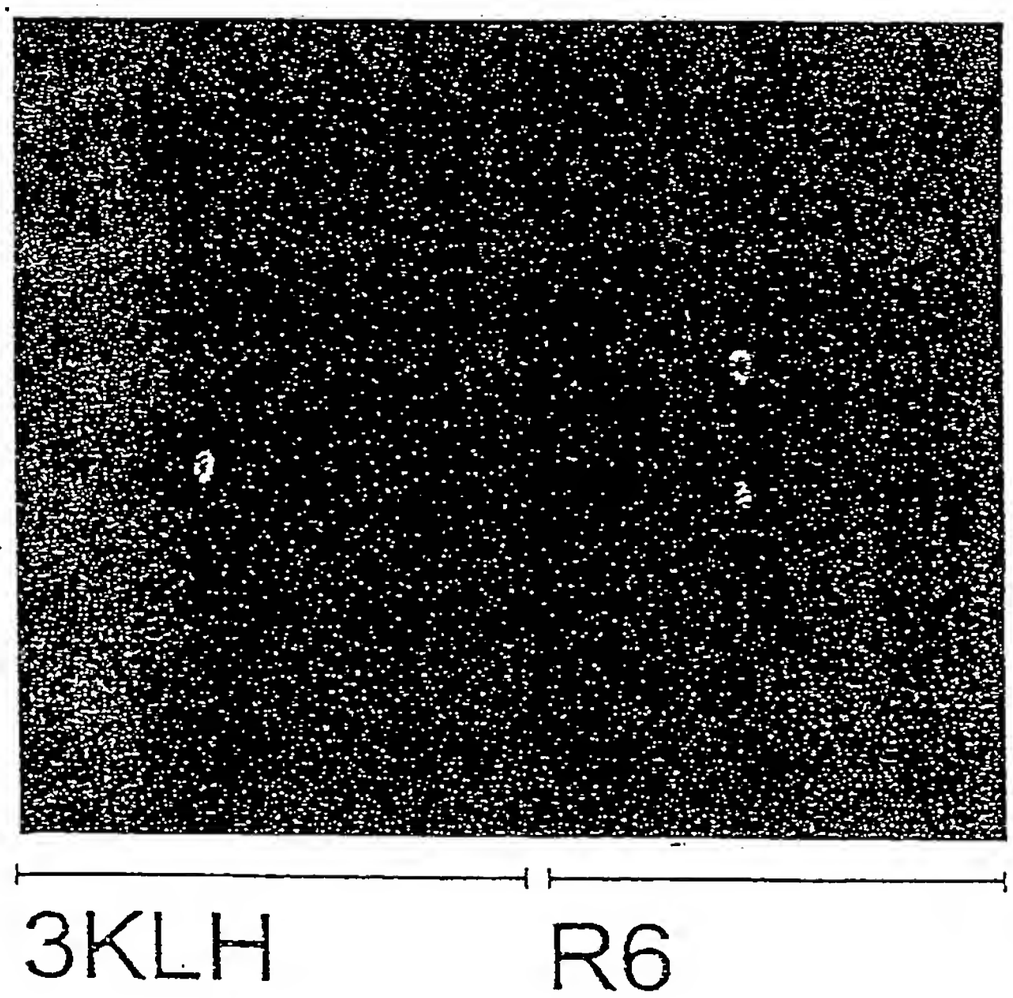


Figure 17

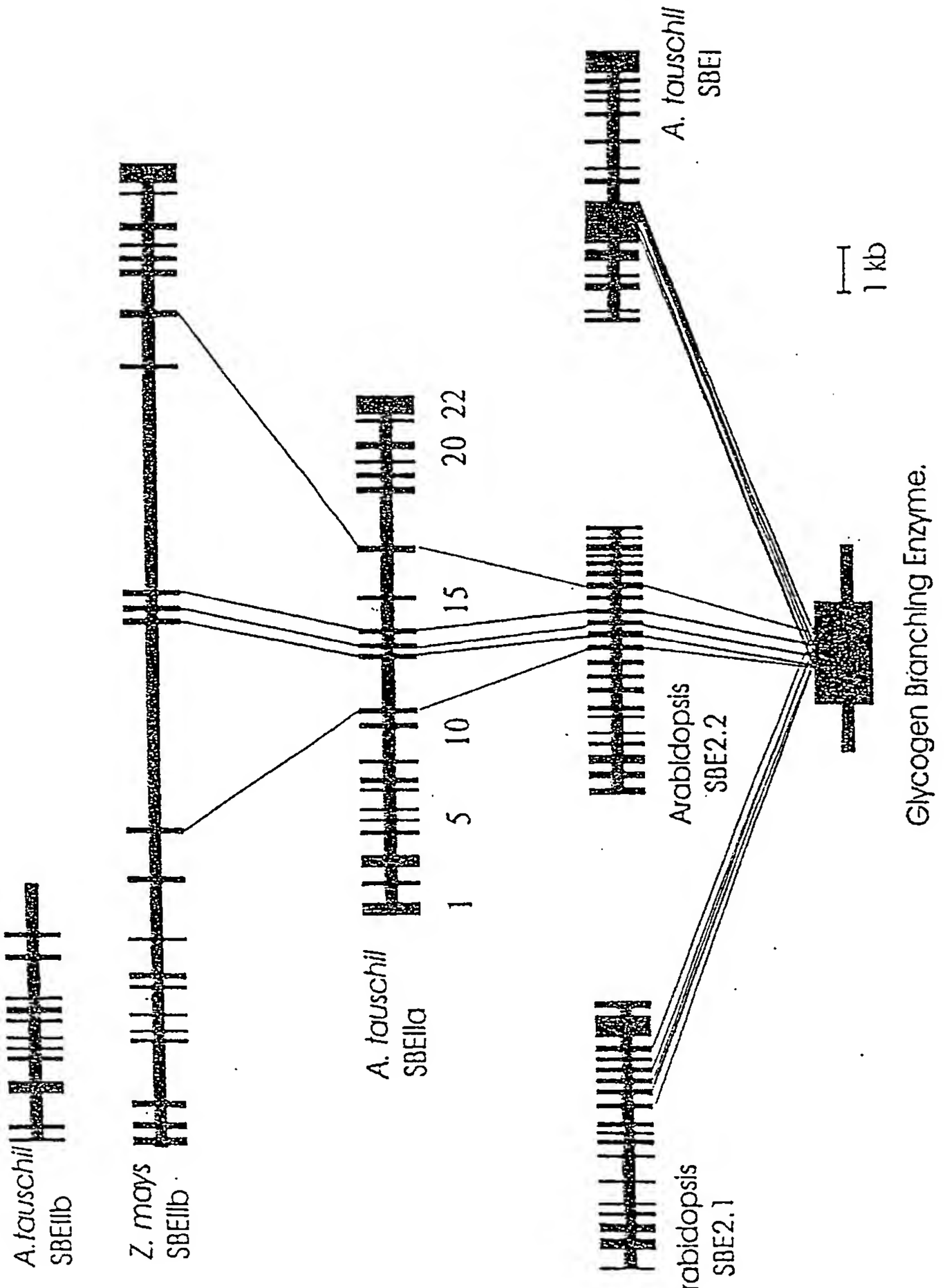

A. tauschii
SBEIIa
1 5 10 15 20 22
A. tauschii
SBEIIb
Z. mays
SBEIIb
Arabidopsis
SBE2.2
Arabidopsis
SBE2.1
1 kb
Glycogen Branching Enzyme.

Figure 18

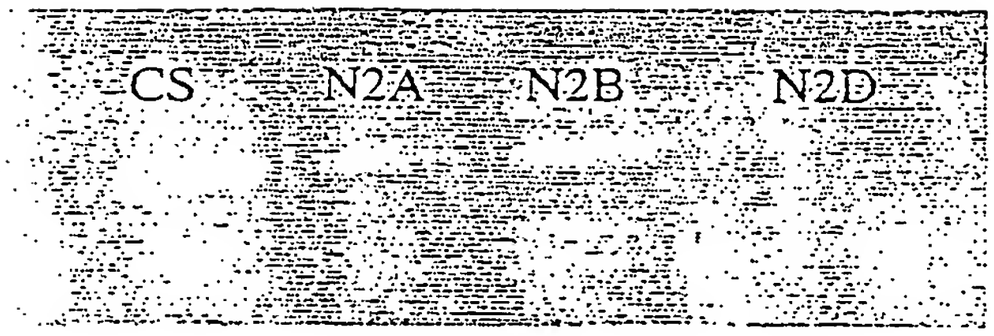


Figure 19

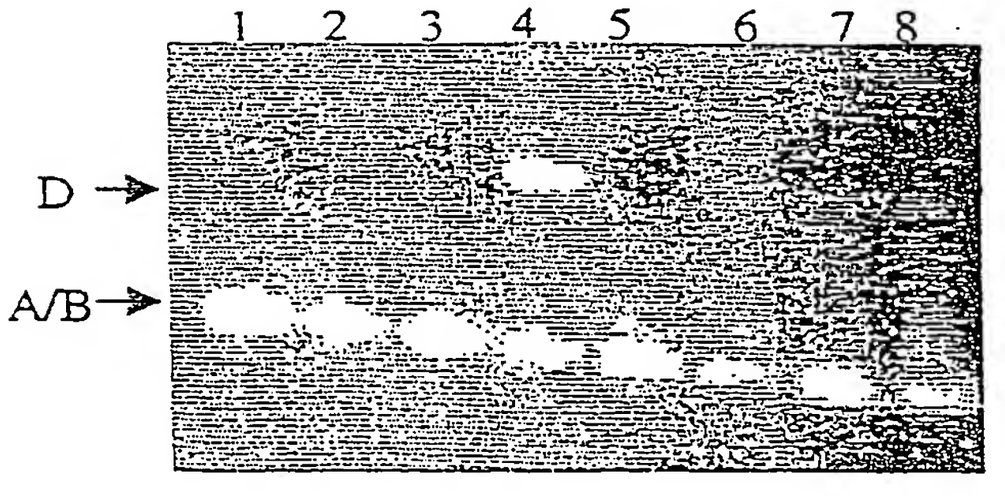


Figure 20

```
                    10         20         30         40         50         60
exon 1/2 A/B*       TGGCGGCGGCGACGGGATGGCTGCGCCGGCATTCGCAGTTTCCGCGGCGGGGCTGGCCC
exon 1/2 A/B        T----GCGGCGACGGGATGGCTGCGCCGGCATTCGCAGTTTCCGCGGCGGGGCTGGCCC
exon 1/2 1 D        CGGCGGCGGCGACGGGATGGCTGCGCCGGCATTCGCAGTTTCCGCGGCGGGGCTGGCCC

exon 1/2 A/B        GGCCGTCGGCTCCTCGATCCGGCGGGGCAGAGCGGAGGGGGCGCGGGGTGGAGCTGCAGT
exon 1/2 A/B        GGCCGTCGGCTCCTCGATCCGGCGGGGCAGAGCGGAGGGGGCGCGGGGTGGAGCTGCAGT
exon 1/2 D          GGCCGTCGGCTCCTCGATCCGGCGGGGCAGAGCGGAGGGGGCGCGGGGTGGAGCTGCAGT

exon 1/2 A/B        CGCCATCGCTGCTCTTCGGCCGCAACAAGGGCACCCGTTCACCCC---------------
exon 1/2 A/B        CGCCATCGCTGCTCTTCGGCCGCAACAAGGGCACCCGTTCACCCC---------------
exon 1/2 D          CGCCATCGCTGCTCTTCGGCCGCAACAAGGGCACCCGTTCACCCCGTAATTATTTGCGCC

exon 1/2 A/B        ----------------------------------------------------------
exon 1/2 A/B        ----------------------------------------------------------
exon 1/2 D          ACCTTTCTCACTCACATTCTCTCGTGTATTCTGTCGTGCTCGCCCTTCGCCGACGACGC

exon 1/2 A/B        ----------------------------------------------------------
exon 1/2 A/B        ----------------------------------------------------------
exon 1/2 D          GTGCCGATTCCGTATCGGGCTGCGGTGTTCAGCGATCTTACGTCGGTTCCCTCCTGGTGT


exon 1/2 A/B          ---------------GTGCCGTCGGCGTCGGAGGTTCTGGATGGCGCGTGGTCATGCGCGC
exon 1/2 A/B          ---------------GTGCCGTCGGCGTCGGAGGTTCTGGATGGCGCGTGGTCATGCGCGC
exon 1/2 D            GGTGATGTCTGTAGGTGCCGTCGGCGTCGGAGGTTCTGGATGGCGCGTGGTCATGCGCGC

exon 1/2 A/B          GGGGGGGCCGTCCGGGGAGGTGATGATCCCTGACGGCG
exon 1/2 A/B          GGGGGGGCCGTCCGGGGAGGTGATGATCCCTGACGGCG
exon 1/2 D            GGGGGGGCCGTCCGGGGAGGTGATGATCCCTGACGGCG
```

Figure 21

```
                    10         20         30         40         50         60         70         80         90
SBE2_AL.DNA      1  TTCTGCCACC ACCGGGAAAT GGACAGCAAA TATACGAGAT TGACCCAACG CTCCGAGACT TTAAGTACCA TCTTGAGTAT CGGTATGCTT    90
SBE2_B.DNA       1  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ------GCTT    90
SBE2_DL.DNA      1  TTCTGCCACC ACCGGGAAAT GGACAGCAAA TATACGAGAT TGACCCAACG CTCCGAGACT TTAAGTACCA TCTTGAGTAT CGGTATGCTT    90

                   100        110        120        130        140        150        160        170        180
SBE2_AL.DNA     91  CGCTTCTATT GTGTGCACTT TAAACTTTAA ATACAATTTA CAGTCTTTGA TAAGATGTGA ATGGCTGCTT GCTGTGACAC AAAACTCTTG   180
SBE2_B.DNA      91  CGCTTCTATT GTGTGCACTT TAAA------ -AACAATTTA CAGTCTTTGA TAAGATGTGA ATGGCTGCTT GCTGTGACAC AAAACTCTTG   180
SBE2_DL.DNA     91  CGCTTCTATT GTGTGCACTT TAAA------ -AACAATTTA CAGTCTTTGA TAAGATGTGA ATGGCTGCTT GCTGTGACAC GAAACTCTTG   180

                   190        200        210        220        230        240        250        260        270
SBE2_AL.DNA    181  AAGTTCGTAG TCACTCTTGT GTGTTCATGG CTCTGAGGTG ACATGGTAAC CGAACAAAAA TAGGAAAGTG GCAAGAACTG CAATGTGAGC   270
SBE2_B.DNA     181  AAGTTCGTAG TCACTCTTGT GTGTTCATGG CTCTGAGGTG ACATGGTAAC CGAACAAAAA TAGGAAAGTG GCAAGAACTG CAATGTGAGC   270
SBE2_DL.DNA    181  AAGTTCGTAG TCACTCTTGT GTGTTCATGG TTCTGAGGTA ACATGGTAAC CGAACAAAAA TAGGAAAGTG GCAAGCACTG CAATGTGAGC   270

                   280        290        300        310        320        330        340        350        360
SBE2_AL.DNA    271  TACCGATAAG CACCCATTGT AATTGGGTAC ACTGATTAAT ATA--TGTCT TGATGGGTTC TATGTTTTTT CAGTATCTAT GCCAATTGAA   360
SBE2_B.DNA     271  TACCGATAAG CACCCATTGT AATTGGGTAC ACTGATTAAT ATA--TGTCT TGATGGGTTC TATGTTTTTT CAGTATCTAT GCCAATTGAA   360
SBE2_DL.DNA    271  TACTGATAAC CACCCATTGT AATTGGGTAC ACTGATTAAT ATATATGTCT TCATGGGCTC TATTTTTTTT CAATATCTAT GCCAATTGAA   360

                   370        380        390        400        410        420        430        440        450
SBE2_AL.DNA    361  CAACAATGC- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------   450
SBE2_B.DNA     361  CAACAATGC- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------   450
SBE2_DL.DNA    361  CAACAATGCT TTGTGGACGG GTGTTCTTTT ACCCTCTTCT TCTATCAATA GATGATATGC ATACTCATGC GTATCCTACA AAAAATTGAA   450

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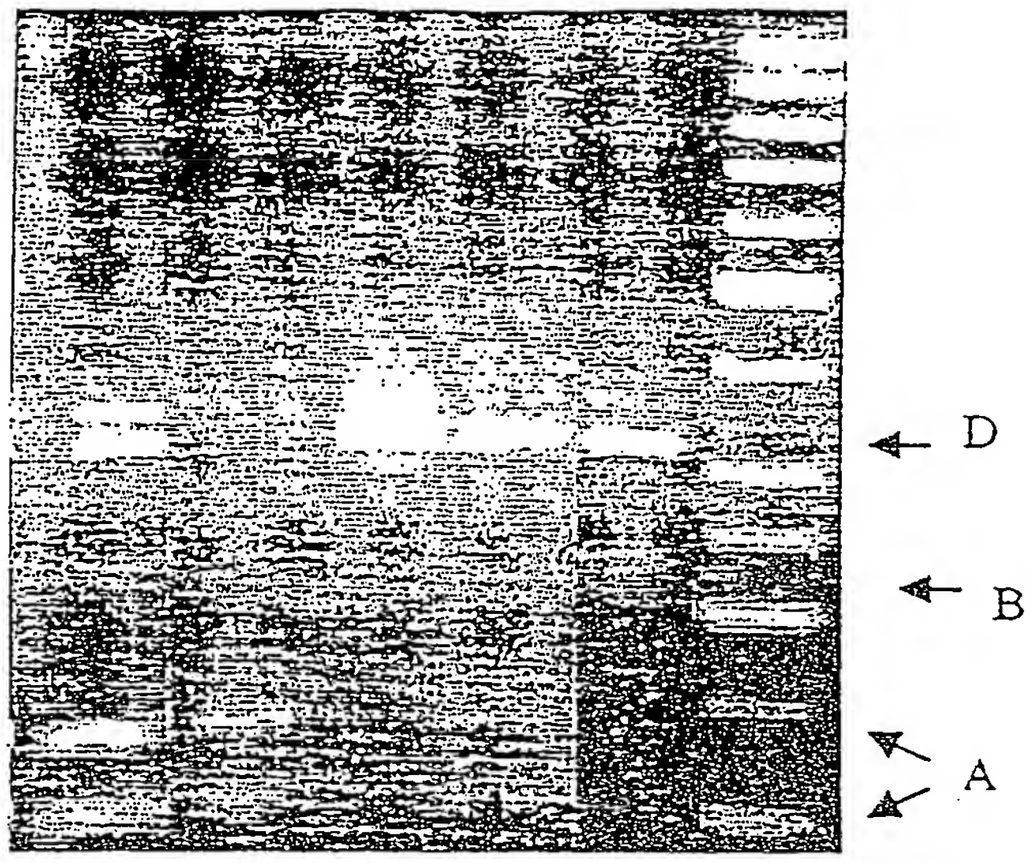

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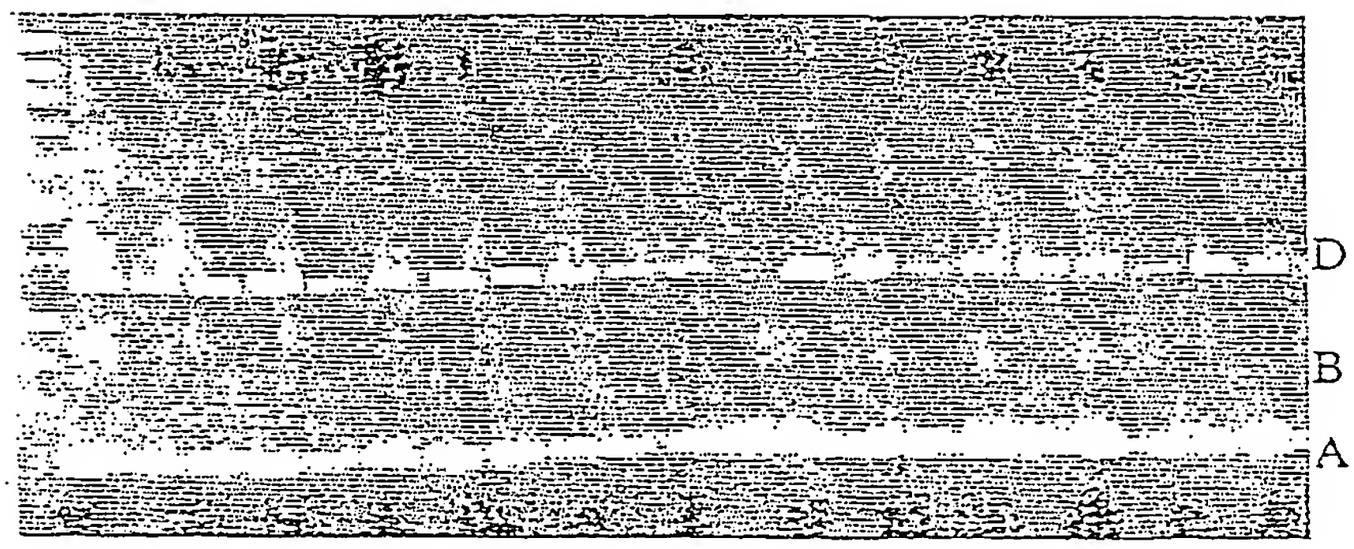

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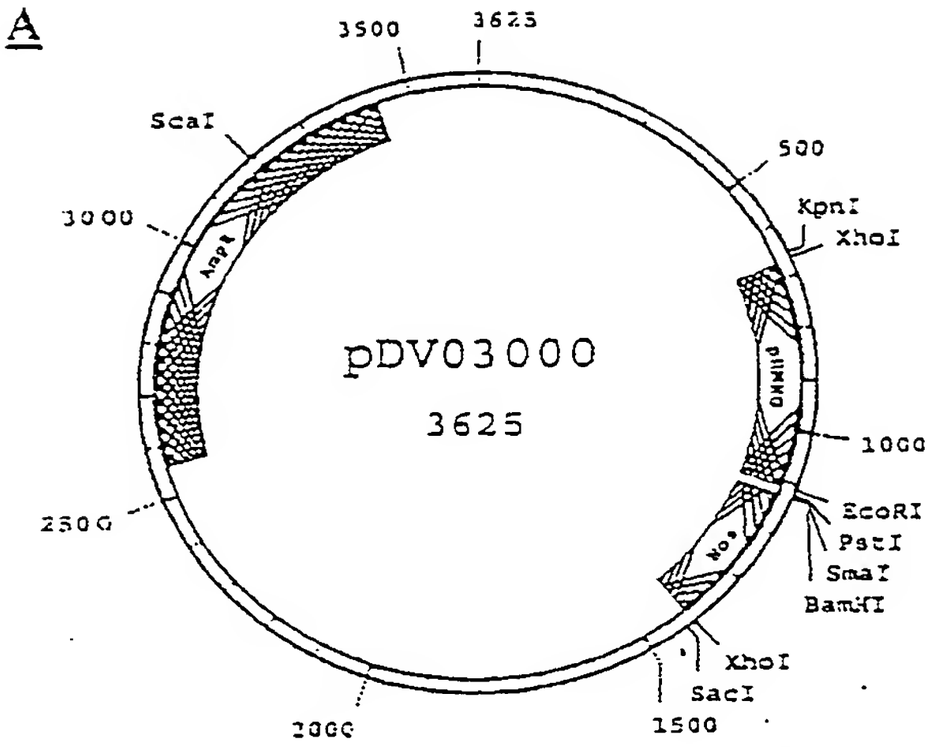

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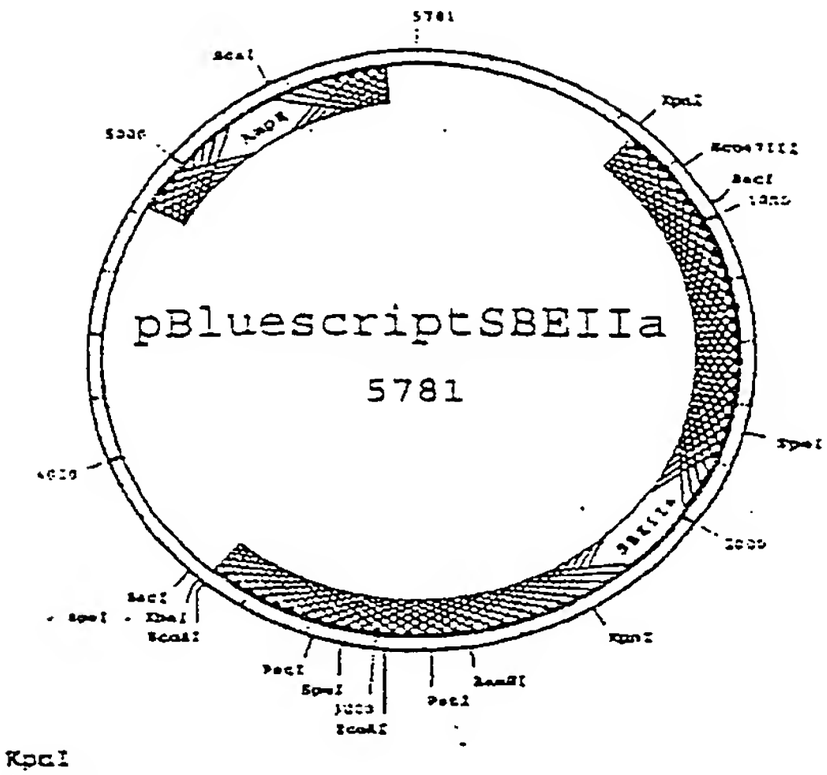

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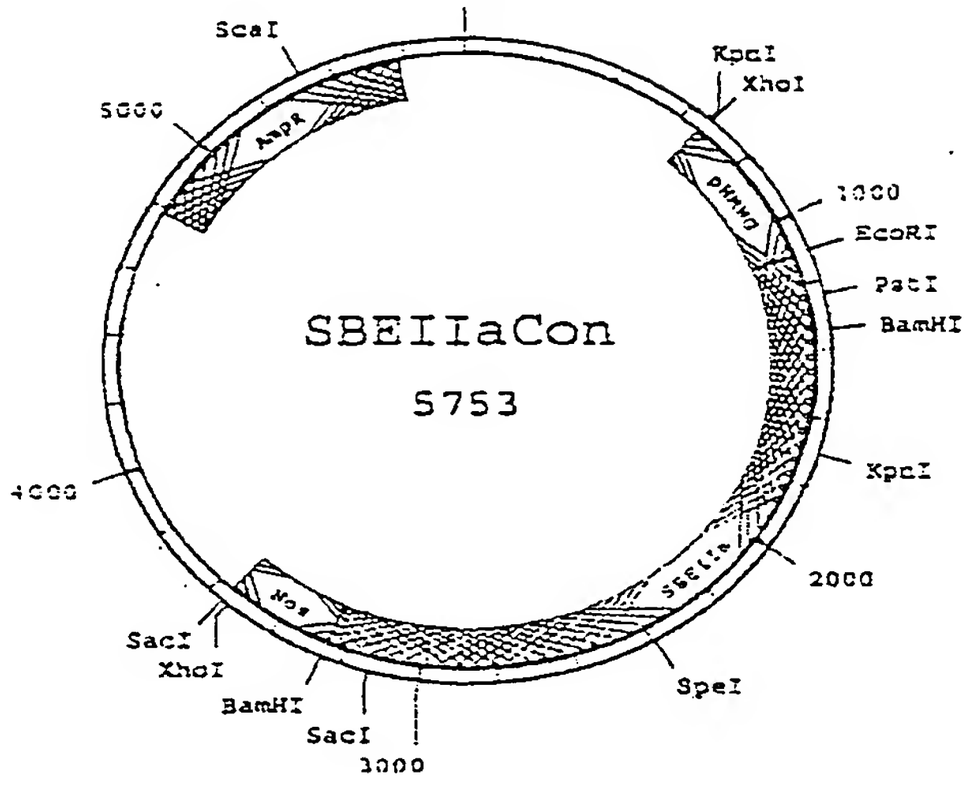

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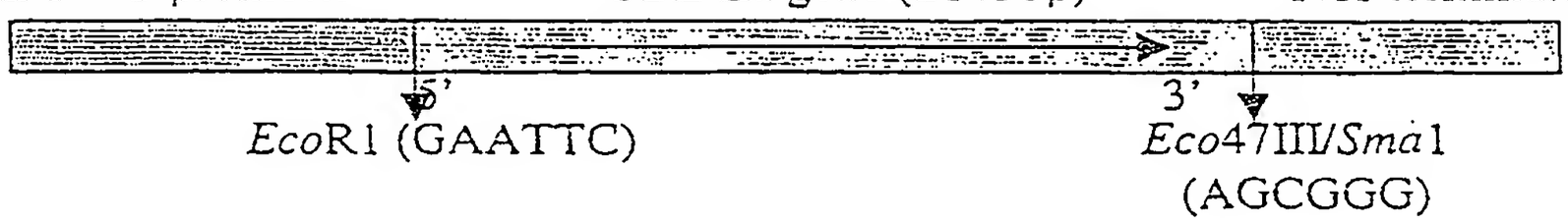

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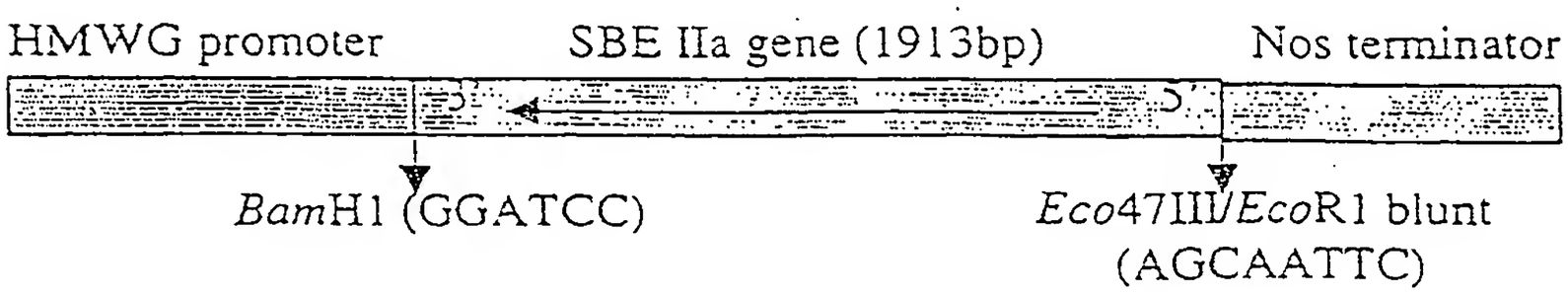

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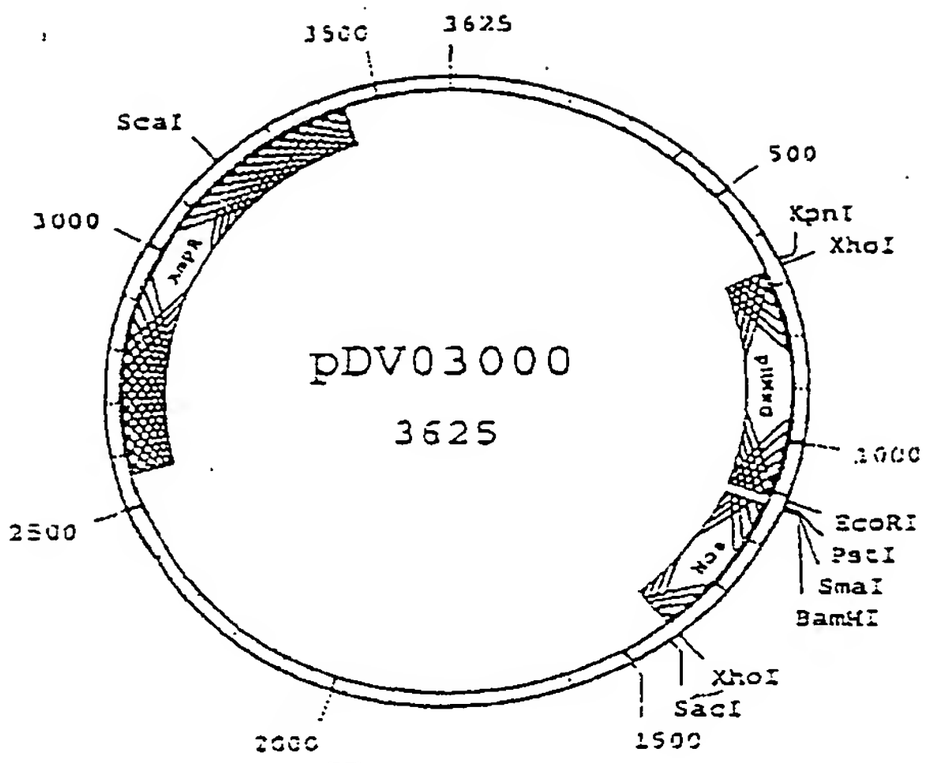

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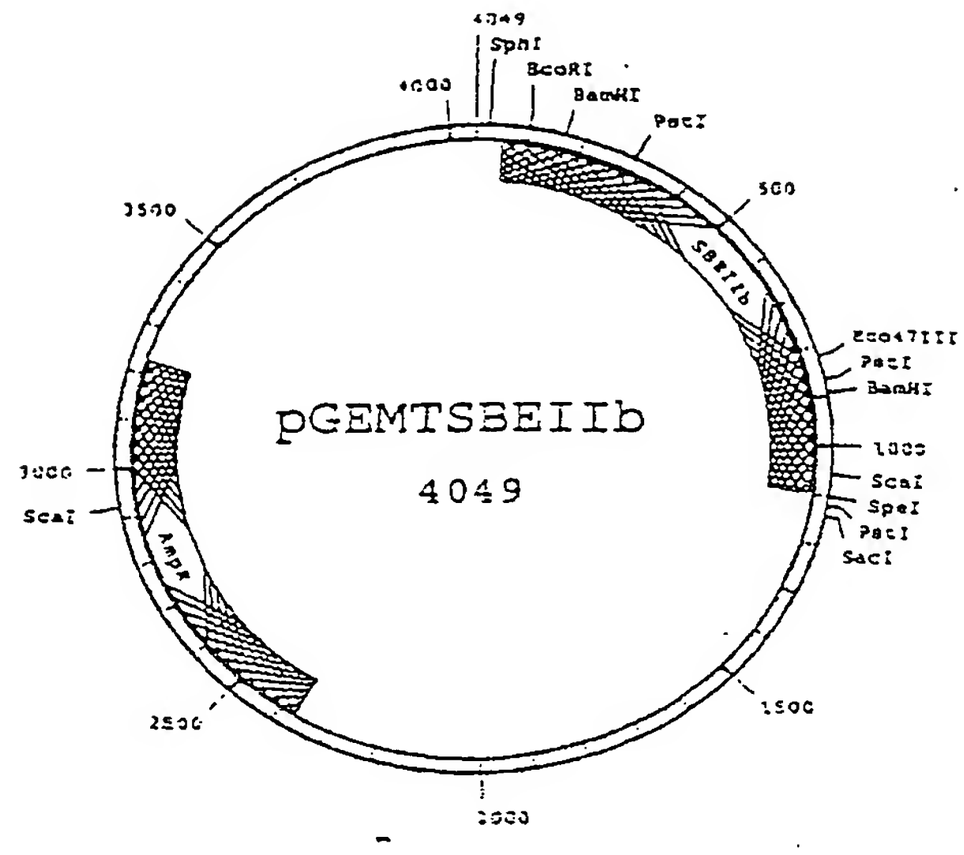

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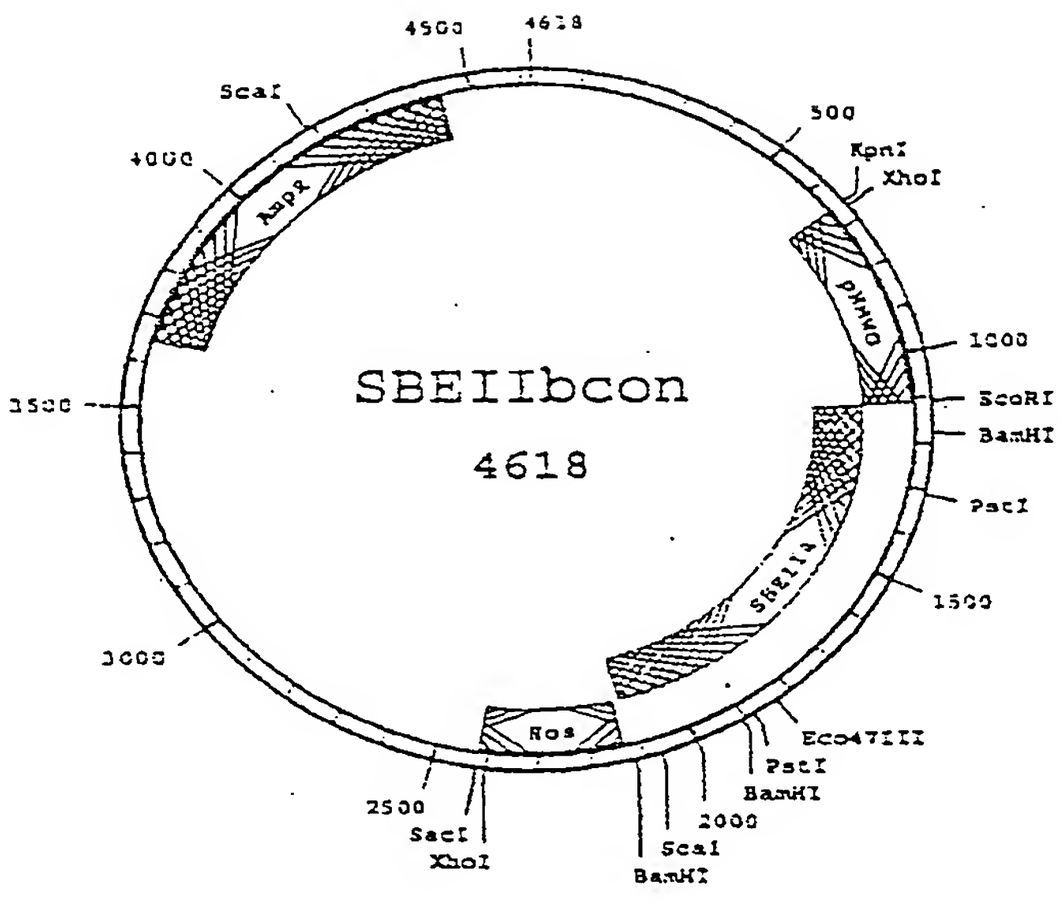

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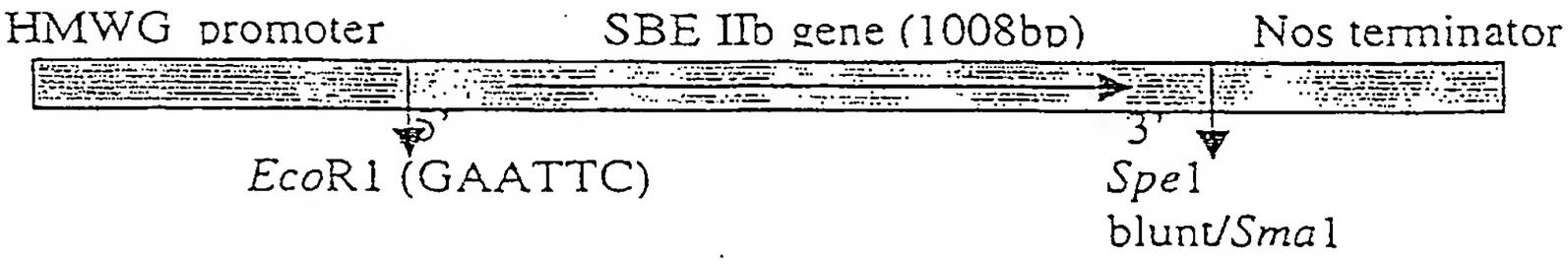

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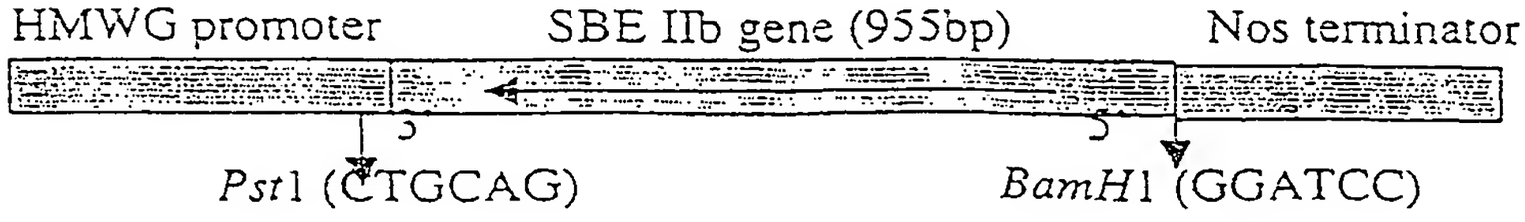
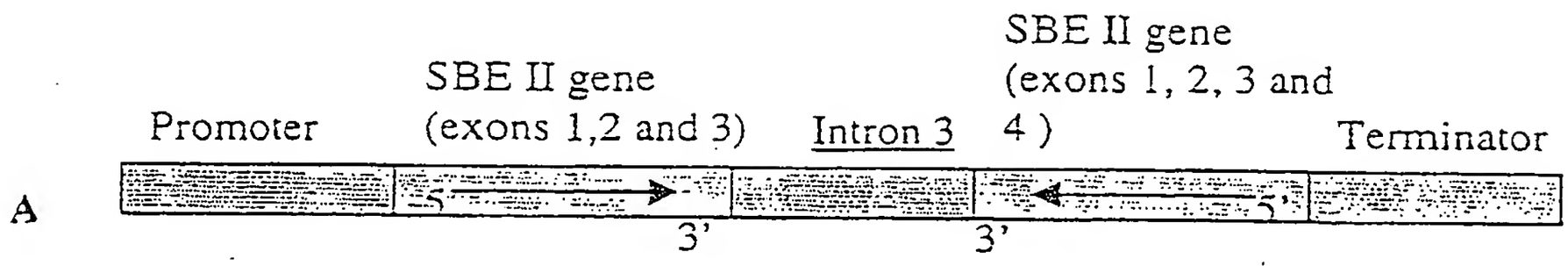


Figure 26

Duplex Construct



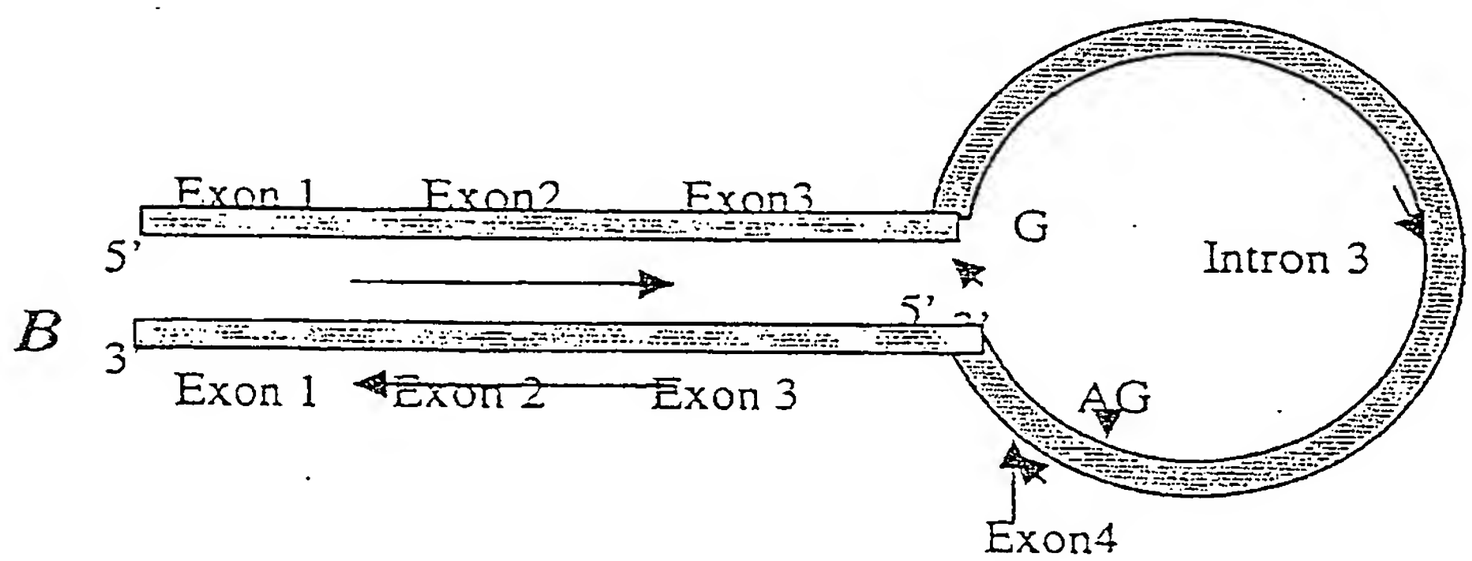


Figure 27

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU01/00175

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C12N 15/29 A01H 5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
DGENE: SEE ELECTRONIC DATA BASE BOX BELOW

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
DNA DATABASES: SEE ELECTRONIC DATA BASE BOX BELOW

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
GENBANK, EMBL: SEQ ID NOS 6 AND 10 DGENE: WHEAT BEIIb PEPTIDE SEQUENCE FROM FIGURE 10

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN C et al "The two genes encoding starch-branching enzymes IIa and IIb are differentially expressed in barley" **Plant Physiol** (1998) 118, pages 37-49 See the entire document	1-52
X	GAO M et al "Evolutionary conservation and expression patterns of maize starch branching enzymes I and IIb genes suggests isoform specialization" **Plant Mol Biol** (1996) 30, pages 1223-32 See the entire document	1-52

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
6 April 2001

Date of mailing of the international search report
19 April 2001

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/00175

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member			
WO	99 14314	AU	89670/98	EP	1012250

END OF ANNEX